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R. H. CHITTENDEN, Ph.D.

Professor of Physiological Chemistry

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The Diastatic Action of Saliva, as Modified by various Conditions, Studied Quantitatively.

By R. H. Chittenden and Herbert E. Smith, M.D.

The chemical changes resulting from the action of unorganized ferments are among the most interesting and important of those which occur in the animal organism. Ferment action plays such an important part in the chemical processes incident to life that definite knowledge of the conditions favorable and inimical to the action of any ferment occurring in the animal body must necessarily be of great physiological value.

Since Leuchs in 1831 discovered the diastatic action of saliva much has been learned regarding this digestive fluid, both as to its chemical action and the nature of the products formed. Still there has been lacking, until recently, definite knowledge of the conditions which influence the diastatic action of the salivary ferment, and it has been the object of the present investigation, taking advantage of previously acquired knowledge, to ascertain the exact influence of those conditions which suggest themselves as being most important in view of the destination of the ferment, and concerning which there has been of late a lack of agreement.

Method used in determining the rate of diastatic action.

In testing the rate of action of the salivary ferment we have in all cases employed quantitative methods, similar in their general nature to those previously used by one of us.* The amount of reducing substances formed by the amylolytic action of the ferment, which for the sake of convenience we have calculated as dextrose, admit of accurate determination by means of the improved Allihn's† method, and thus enable us to give a concise expression of the relative diastatic action, even in those cases where the differences are very slight. As recent experiments‡ have plainly indicated, the ultimate product of the dias-

* Chittenden and Griswold, Amer. Chem. Jour., iii, 305. Chittenden and Ely, ibid, iv, 107.

† Zeitschrift für analytische Chemie, 22 Jahrgang, p. 448.

‡ v. Mering and Musculus, Zeitschrift für physiologische Chemie, i, 395. O. Sullivan and E. Schultze, Berichte d. deutsch. Chem. Gesell., vii, 1047. Musculus and Gruber, Zeitschrift für physiolog. Chemie, ii, 177. v. Mering, Zeitschrift für physiolog. Chem., v, 196.

tatic action of ptyalin is dextrose. The sugar intermediate between this body and the dextrins, and which is formed in much larger quantity is maltose, with a relative reducing power of 66 as compared with dextrose, 100; while the achroodextrins and other intermediate products have very small reducing power. Consequently the reducing power of a digestive mixture must necessarily express the relative diastatic action of the ferment present, since increased action means an increased formation of reducing bodies, of which the final product has the highest reducing power. In this connection it is well to remember that diastase and ptyalin both convert only a limited quantity of starch into sugar or reducing bodies,* and that no matter how great the excess of ferment or the length of time the action is continued, the percentage of starch changed into sugar does not ordinarily exceed 53 per cent.† The general method employed in our work for testing the diastatic action of saliva was as follows: the volume of the digestive mixture was in every instance 100 c. c.; the amount of starch‡ present 1 or 2 grams, previously boiled in a definite amount of water; the temperature of digestion 38–40° C.; the length of time generally 30 minutes. When the digestion was finished, diastatic action was at once stopped by boiling the mixture; when cold, the mixture was diluted with distilled water to 200 c. c. and filtered; 25 c. c. of the filtrate or one-eighth of the entire fluid was then precipitated with Fehling's solution according to Allihn's§ data and method; the reduced copper was filtered through an asbestos filter in a small weighed glass tube and ignited directly in a current of hydrogen gas and weighed as metallic copper. By means of Allihn's tables of reduction equivalents the corresponding amount of sugar, calculated as dextrose, is easily obtained, from which the percentage amount of starch converted into reducing bodies can be computed, calling dextrose $C_6H_{12}O_6$ and the starch $C_6H_{10}O_5$.‖ The following experiment illustrates the accuracy of the method and the reliance which can be placed upon it; two solutions of 100 c. c., each containing 2 grams of starch and 4 c. c. of filtered

* Schulze and Märker, Chem. Centralb. 1872, 823. Chittenden and Ely, Amer. Chem. Jour., iv, 120.

† Musculus and v. Mering, Zeitschrift für Physiolog. Chem., ii, p. 415.

‡ The starch was exactly neutral; made so by long and thorough washing with pure water.

§ Loc. cit.

‖ The actual amount of starch changed is, however, somewhat greater than would appear by this equation, since, as has already been mentioned, considerable of the sugar formed is maltose, which has only two-thirds the reducing power of dextrose.

saliva were warmed at 40° C. for 4 hours, then examined with the following results :

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
I.	0·1530 gram.	0·6248 gram.	28·13 per cent.
II.	0·1523	0·6216	27·91

Relation of dilution to diastatic action.

It is a fact well understood that the chemical action of a ferment is out of all proportion to the amount of ferment present; indeed, a given solution of a ferment can be diluted again and again without any marked difference in its chemical activity, or at least none at all proportionate to the degree of dilution. It is only when dilution has been carried to the extreme limit that the relative amylolytic power of the mixture can be taken as a measure of the amount of ferment present.

The following experiments illustrate the foregoing statement. Each digestive mixture was 100 c. c. in volume, and was warmed at 40° C. for 30 minutes. The only variations in the different mixtures consisted in the amount of saliva and starch.

SERIES I.

With 1 per cent. starch.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
20 c.c. saliva,	0·0951 gram.	0·3872 gram.	34·87 per cent.
10	0·0878	0·3584	32·26
5	0·0809	0·3296	29·67
4	0·0710	0·2904	26·14
3	0·0635	0·2608	23·48
2	0·0452	0·1880	16·92
1	0·0178	0·0792	7·23
½	0·0080	0·0408	3·66

With 2 per cent. starch.

20 c.c. saliva,	0·1784 gram.	0·7304 gram.	32·87 per cent.
10	0·1641	0·6704	30·18

SERIES II.

a. with 1 per cent. starch and 30 minutes at 40° C.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c.c. saliva,	0·0721 gram.	0·2944 gram.	26·50 per cent.
2	0·0480	0·1992	17·93
1	0·0211	0·0920	8·28

b. with 2 per cent. starch and 30 minutes at 40° C.

4 c.c. saliva,	0·1006 gram.	0·4088 gram.	18·40 per cent.
2	0·0408	0·1704	7·67
1	trace		

c. with 1 per cent. starch and 10 minutes at 40° C.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c.c. saliva,	0·0573 gram.	0·2352 gram.	21·15 per cent.
2	0·0213	0·0928	8·35
1	0·0091	0·0456	4·11

SERIES III.

a. with 1 per cent. starch and 30 minutes at 40° C.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c.c. saliva,	0·0650 gram.	0·2664 gram.	23·98 per cent.
2	0·0313	0·1336	12·01
1	0·0139	0·0644	5·79

b. with 2 per cent. starch and 30 minutes at 40° C.

4 c.c. saliva,	0·0769 gram.	0·3136 gram.	19·26 per cent.
2	0·0250	0·1080	4·86
1	0·0103	0·0504	2·27

c. with 2 per cent. starch and 4 hours at 40° C.

4 c.c. saliva,	0·1530 gram.	0·6248 gram.	28·13 per cent.
2	0·1058	0·4312	19·41
1	0·0681	0·2784	12·53

From these results it is seen that only when the dilution of normally alkaline saliva is as 1 : 50 or 100 does the diastatic action at all correspond to the amount of ferment present. The same is to be noticed in Grützner's* experiments, where the principle employed by Gruenhagen in the estimation of pepsin was used; the amount of starch dissolved by the saliva being directly proportional to the amount of ferment, only when very small quantities of saliva were employed and the time limited to 10 or 15 minutes. Increasing the amount of starch beyond 1 per cent. tends to diminish somewhat the amount of sugar formed in a given time, when the dilution of the saliva is as 1 : 50 or 100, which fact agrees well with what we already know concerning the influence on ferment action of the clogging of digestive fluids in general by the products of digestion, or by the substance to be digested; series III, *a* and *b*. Increasing the length of time for the ferment to act, however, causes a corresponding increase in the amount of sugar formed, as is well seen in series III, c. It would not be at all impossible therefore by suitable dilutions to use this method as a means of determining the relative amounts of ptyalin present in different salivary or pancreatic secretions. The following results, taken from those already given, in addition to others, lends favor to this view. All the experiments were made in

* Pflüger's Archiv der Physiologie, xii, p. 294.

the usual way, and the results are expressed in percentage of starch converted into sugar.

	1	2	3
2 c.c. saliva,	12·01 per cent.	8·35 per cent.	4·73 per cent.
1	5·79	4·11	2·21
	4	5	6
1 c.c. saliva,	6·93 per cent.	26·81 per cent.*	24·00 per cent.*
½	3·56	13·72	11·34

The degree of dilution to be employed depends, of course, upon the amount of ferment present. We have usually diluted the saliva 5 or 10 times, and then added an amount of the diluted fluid corresponding to 0·5–2·0 c. c. of saliva, which in the 100 c. c. of digestive mixture makes a dilution of from 50 to 200. As we shall have occasion to state later on, neutralized saliva needs even greater dilution. The method certainly appears as advantageous as that proposed by Dr. Roberts† a few years ago, and has the advantage of giving gravimetric results, instead of being dependent upon the disappearance of a shade of color. In using the method with different solutions it will always be found necessary to exactly neutralize the ptyalin-containing solutions, before diluting them, since variations of alkalinity, even though infinitesimal in amount, may produce discordant results. Moreover, it is better to warm the ptyalin solution with the starch for not longer than 30 minutes.

The amount of dilution which saliva will endure and still show diastatic action depends naturally upon the amount of ptyalin present in the secretion and also upon the reaction of the fluid, whether it be alkaline or neutral. The following series of experiments show the average of our results on this point.

SERIES IV.

Normally alkaline saliva, 1 per cent. starch.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
1 c.c. saliva,	0·0152 gram.	0·0704 gram.	6·33 per cent.
½	0·0057	0·0272	2·44
¼	0·0037	0·0176	1·59
$\frac{1}{10}$	trace	less than 1 per cent. of starch converted.	
$\frac{1}{20}$‡	trace		

It is thus seen that when the dilution is as 1 : 250, an appreciable

* Neutralized saliva.

† William Roberts: Jahresbericht für Thierchemie, 1881, 290.

‡ To ensure greater accuracy the saliva was diluted ten times and amounts of the diluted fluid added corresponding to the above.

amount of starch is converted into sugar in 30 minutes at 40° C. Even with a dilution of 1:1000 or 2000, a recognizable amount of sugar is formed under these conditions. This degree of dilution, however, cannot be considered as being the limit at which diastatic action will show itself, for with even greater dilutions, the starch is converted into soluble modifications, colored blue by iodine, without giving any recognizable amount of reducing substance; that is, in one-eighth of the digestive mixture. Longer continued action at 40° C. might yield some reducing substance; it would seem, however, from our experiments, that when a certain degree of dilution is reached, the action of the small amount of ferment in contact with the larger amount of starch (1 gram) is devoted exclusively to converting the granulose into soluble starch or other like body with non-reducing action. This agrees with the results obtained by Grutzner,* who found that the nature of the products obtained by the action of ptyalin was dependent upon the intensity of the ferment action; with a small amount of ferment, erythrodextrin was the main product, while with a large amount of ferment, sugar was mainly formed. Diminishing the amount of starch in large dilutions of saliva tends, as might be expected, to increase the amount of sugar formed.

Comparison of the diastatic action of neutralized and normally alkaline saliva.

Human mixed saliva, when freshly secreted, almost invariably possesses a distinct alkaline reaction. Some time ago one of us published a series of experiments† on this point, in which it was shown that the average alkalinity of 51 samples of human mixed saliva, expressed as sodium carbonate, was 0·080 per cent. The extreme variations of alkalinity in the saliva from 14 individuals amounted to 0·085 per cent. calculated as sodium carbonate (0·144–0·059 per cent).

We have had occasion to make determinations of alkalinity in 15 additional samples of saliva, all collected by one person. We give the results here, as affording additional data regarding the average alkalinity of this secretion. The alkalinity is calculated, as heretofore, in the form of sodium carbonate.‡ The indicator used was delicate litmus paper.

* Pflüger's Archiv der Physiologie, xii, p. 297.

† Chittenden and Ely, Amer. Chem. Jour., iv, 329.

‡ Undoubtedly the alkaline reaction of saliva is due in part to alkaline phosphates, and probably the precentages given are only an approximation to the truth.

Filtered saliva.	0·2 per cent. HCl used in neutralizing.	Amount of alkalinity.
20 c.c.	6·25 c.c.	0·091 per cent.
40	10·70	0·078
40	12·00	0·087
25	9·10	0·116
20	6·00	0·087
20	6·25	0·091
20	6·75	0·098
20	5·30	0·077
40	12·50	0·091
20	7·00	0·102
40	12·20	0·088
20	7·80	0·113
20	6·80	0·099
20	8·30	0·120
20	7·60	0·110

Average alkalinity of the 15 samples, 0·097 per cent.

It was demonstrated some time ago by one of us* that neutralized saliva had as great a diastatic power as the unneutralized or normally alkaline secretion. In fact, the single result which we recorded plainly indicated a greater diastatic power on the part of neutralized saliva, since from the digestion with the normally alkaline fluid, one-tenth of the mixture gave 0·0905 gram metallic copper, while the same quantity of the saliva neutralized, gave under like conditions 0·0943 gram copper; thus showing that the alkaline saliva had converted 41·58 per cent. of the starch into sugar, while the same quantity neutralized had changed 43·28 per cent. In these two experiments, however, the amount of saliva used was large, being one-fourth of the entire digestive mixture, viz., 25 c.c.

Recently Langley and Eves† have made the statement that "neutralized saliva converts starch into sugar much more actively than unneutralized saliva," without, however, giving any data. These are the only two statements recorded, bearing on the relative diastatic action of the neutralized and normally alkaline secretion.

Our experiments, however, show that there is a very great difference in the action of ptyalin in neutralized and unneutralized saliva; a difference which is more manifest when the saliva is greatly diluted and seemingly out of all proportion to the amount of alkali present, in cases where the dilution is 1 : 100 or more. The following experiments show the amount of difference.

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* Chittenden and Ely, Amer. Chem. Jour., iv, 112.

† On certain conditions which influence the amylolytic action of saliva. Journal of Physiology, vol. iv, No. 1.

SERIES V.

The saliva used in this series contained 0·091 per cent. alkali, calculated as sodium carbonate:

20 c.c. of saliva were diluted to 100 c.c. and used in *a*.

20 c.c. of the same saliva were neutralized and then diluted to 100 c.c. and used in *b*.

a. normally alkaline saliva.

	Wt. Cu in one-eighth.	Total amount of sugar.	Starch converted into sugar.
4 c.c. saliva,	0·0652 gram.	0·2672 gram.	24·05 per cent.
2	0·0282	0·1208	10·87
1	0·0094	0·0464	4·17

b. neutralized saliva.

4 c.c. saliva,	0·0867 gram.	0·3536 gram.	31·83 per cent.
2	0·0730	0·2984	26·72
1	0·0373	0·1560	14·04

The difference in diastatic action in this instance, particularly where the dilution is as 1 : 50 and 100, is very great, yet in the case of the greatest dilution of the unneutralized saliva the alkalinity of the digestive mixture is but 0·00091 per cent. calculated as alkaline carbonate. Moreover, there is a greater proportional diminution of diastatic action in this case, and also in the next greatest dilution where the amount of alkalinity is 0·00182 per cent., than in the presence of 0·00364 per cent.; a fact due either to the greater susceptibility of the ferment to alkaline carbonate in a dilute solution or else to some modifying influence of the larger amount of albuminous matter present, a point which we shall return to later.

Carrying the dilution of the saliva still further we find that the difference between the diastatic action of the neutralized and unneutralized fluid shows itself to the limit of decisive diastatic action.

SERIES VI.

This sample of saliva contained 0·116 per cent. of alkali calculated as sodium carbonate. The percentages of starch converted into sugar during 30 minutes at 40° C. alone are given.

Amount of saliva.	Alkali in the 100 c.c. of digestive mixture.	Alkaline saliva.	Neutralized saliva.
1 c.c.	0·00116 per cent.	6·33 per cent.	16·34 per cent.
$\frac{1}{2}$	0·00058	2·44	6·62
$\frac{1}{4}$	0·00029	1·54	2·07
$\frac{1}{10}$	0·00011	trace	result lost.
$\frac{1}{20}$	0·00005	trace	1·25 per cent.

Thus in a dilution of 1 : 2000 in the case of neutralized saliva, dias-

tatic action is still sufficiently pronounced to convert 1·25 per cent. of the starch into sugar during 30 minutes warming at 40° C.

The above results, indicative of such a marked susceptibility of the ferment in a dilute solution to the action of the alkali naturally present in saliva, suggest the possibility of there being a direct connection between the alkalinity of the natural secretion and its diastatic power. While the results already given plainly indicate that very slight changes in the alkalinity, everything else being equal, materially modify the diastatic power of the fluid, the amount of ferment itself, as well as the amount of proteid matter, may vary in different salivas so much as to counterbalance the direct influence of changes in alkalinity.

This, the results of our experiments seem to indicate, as we have been unable to trace out any direct connection between the natural variations of alkalinity and diastatic action.*

Influence of different percentages of sodium carbonate on the diastatic action of saliva.

In 1882, while studying the influence of peptones on the diastatic action of alkaline saliva,† data were then obtained showing a constant diminution of diastatic action in the presence of sodium carbonate; the conversion of starch into sugar being diminished in proportion as the percentage of alkali was increased. The digestions at 40° C. were then continued for 45 minutes and the ferment was present in large amount, 25 of the 100 c.c. of digestive mixture being undiluted, unneutralized saliva, thus making a very powerful diastatic fluid. We give the data then obtained in the percentage of starch or glycogen converted into sugar.

a. Influence of 0·05 per cent. sodium carbonate.

	Saliva alone.	Saliva + Na_2Co_3 = 0·05%	Difference.
Glycogen,	28·68 per cent.	20·20 per cent.	8·48 per cent.

b. Influence of 0·15 per cent. sodium carbonate.‡

	Saliva alone.	Saliva + Na_2Co_3 = 0·15%.	Difference.
Starch,	40·23 per cent.	17·48 per cent.	22·75 per cent.
"	37·15	14.72	22·43
"	37·55	15·48	22·07
"	38·36	13·57	24·79
Glycogen,	28·68	9·40	

* Compare Chittenden and Ely, Amer. Chem. Jour., iv, 329.

† Chittenden and Ely, Amer. Chem. Jour., iv, 121.

‡ The alkalinity is somewhat greater, owing to the unneutralized alkali of the saliva.

c. Influence of 0·30 per cent. sodium carbonate.

	Saliva alone.	Saliva + Na_2Co_3 = 0·30 %.	Difference.
Starch,	40·27 per cent.	10·83 per cent.	29·44 per cent.
"	40·23	9·87	30·36
"	37·15	9·52	27·63
"	38·80	9·79	29·01
"	37·55	10·01	27·54
"	38·36	9·60	28·76
Glycogen,	29·11	6·93	

The action of the sodium carbonate is here very marked and very constant.

We have repeated this series of experiments in part, varying the conditions only by using neutralized saliva, so that the percentages of alkali present might be exact.*

SERIES VIII.

Per cent. Na_2Co_3.	Starch converted.	Difference.
0	41·16 per cent.	
0·005	39·47	1·69 per cent.
0·025	34·84	6·32
0·050	29·81	11·35
0·150	17·88	23·28
0·300	10·88	30·28

It is evident from these results that the presence of a definite percentage of sodium carbonate will produce approximately a constant diminution in the diastatic action of the saliva. This result, however, is constant only when the saliva acts in the above dilution. Diminish the amount of ferment—or rather dilute the saliva—and then the above percentages of alkali produce quite a different result. The above results were obtained where the dilution of the saliva was as 1 : 4. Adding now neutralized saliva to the alkaline mixtures of starch and water in such proportion that 10 c.c. of the original saliva are present in 100 c.c. of digestive mixture; i. e., a dilution of 1 : 10, the results are different.

The following figures were obtained with the above dilution, the mixtures being warmed at 40° C. for 30 minutes.

SERIES IX.

Per cent. Na_2Co_3.	Wt. Cu in one-eighth.	Total amt. sugar formed.
0	0·0998 gram.	0·4064 gram.
0·005	0·0898	0·3664
0·025	0·0437	0·1816
0·050	0·0277	0·1184
0·100	0·0182	0·0808
0·300	0·0105	0·0504
0·500	0·0091	0·0448

* The standard solutions of sodium carbonate were made from the chemically pure, anhydrous salt.

These figures lead to the following percentages of starch converted into sugar under the different degrees of alkalinity.

Per cent. Na_2Co_3.	Starch converted.	Difference.
0	36·57 per cent.	
0·005	32·98	3·59 per cent.
0·025	16·35	20·22
0·050	10·66	25·91
0·100	7·27	29·30
0·300	4·53	32·04
0·500	4·03	32·54

By comparing the two preceding columns of differences it is very manifest that the alkaline carbonate has a much greater retarding action on the more dilute saliva than on the stronger solution; very noticeably so in the mixtures containing 0·025 and 0·050 per cent. of the alkaline salt.

By diluting neutralized saliva still more, and then using quantities of the fluid equal to 2 c.c. of the original saliva, making in the 100 c.c. of digestive mixture a dilution of 1 : 50, even 0·005 per cent. of sodium carbonate is sufficient to retard the diastatic action of the ferment almost completely; thus in one experiment with the above amount of saliva in the presence of 0·005 per cent. sodium carbonate, but 4·03 per cent. of the starch was converted into sugar in 30 minutes at 40° C., while the same amount of saliva alone converted 27·08 per cent. of the starch into sugar. By increasing the percentage of alkaline carbonate diastatic action was stopped completely.

It is thus evident that the percentage of alkaline carbonate which absolutely or to a certain extent hinders the diastatic action of saliva can be designated only for a definite mixture, and not in a general sense. Langley and Eves* state that sodium carbonate of 0·0015 per cent. causes a retardation in the action of ptyalin; our experiments with unneutralized saliva diluted, plainly show that even much smaller percentages of alkalinity may decidedly retard the action of the ferment, while in similarly diluted saliva 0·005 per cent. of sodium carbonate may prevent diastatic action almost entirely.

Again Langley and Eves† state that the "amylolytic action of saliva becomes less the more alkaline salt there is in the solution, the rate of decrease is, however, slow compared with that which occurs when hydrochloric acid is added in similarly increasing quantities." The rate of decrease, however, as our experiments plainly show, is dependent greatly upon the amount of dilution.

* Journal of Physiology, vol. iv, No. 1. † Ibid.

Destruction of salivary ptyalin by sodium carbonate.

To how great an extent is the retarding influence of sodium carbonate due to destruction of the ferment? Langley and Eves* state that "sodium carbonate has a very slight destructive action on ptyalin, its retarding power is out of all proportion to its power of destruction."

The following experiments demonstrate its manner of action under various conditions.

SERIES X.

70 c.c. of filtered saliva (the same saliva as used in Series IX), were exactly neutralized with 0·2 per cent. HCl and diluted to 140 c.c.

The following mixtures were then prepared:

	1	2	3	4	5
Diluted saliva,	20 c.c.	20 c.c.	20 c.c.	20 c.c.	20 c.c.
Na_2CO_3 sol.,	0	20 " 0·1%	10 " 0·6%	20 " 0·6%	20 " 1·%
H_2O	20 "	0	10 "	0	0
Per cent. Na_2Co_3,	0	0·05	0·15	0·30	0·50

These were warmed at 40° C. for 30 minutes, then neutralized with the amounts of dilute acid given below, water and starch added, and the mixtures again warmed at 40° C. for 30 minutes.

	1	2	3	4	5
HCl 0·2 per cent.,	0	6·88 c.c.	20·6 c.c.	41·3 c.c.	68·8 c.c.
Starch + H_2O,	60 c.c.	53·20	39·4	18·7	20·0
	100 c.c.	100 c.c.	100 c.c.	100 c.c.	128·8 c.c.

	Wt. Cu in one-eighth.	Total amount sugar.	Starch converted.
1	0·0998 gram.	0·4064 gram.	36·59 per cent.
2	0·0991	0·4032	36·30
3	0·0992	0·4040	36·40
4	0·0474	0·1968	17·71
5	0·0278	0·1192	10·73

In the above digestive mixtures the ultimate dilution of the saliva is the same as in series IX, 1 : 10, and being the same saliva the above results are directly comparable with those of series IX. Warming saliva of the above strength with 0·05 and 0·15 per cent. sodium carbonate for 30 minutes causes no destruction of the ptyalin whatever, as the results of experiments 2 and 3 indicate, consequently any diminished diastatic action in the presence of the above percentages of alkaline carbonate must be due to a simple retardation in

* Journal of Physiology, vol. iv, No. 1.

the action of the ferment and not to its destruction. On the other hand, 0·3 and 0·5 per cent. sodium carbonate under like conditions and with the same strength of saliva cause a marked destruction of the ferment, as the results of experiments 4 and 5 plainly show.

We have repeated the above series of experiments with a saliva, neutralized and diluted 5 times, using in each experiment 10 c.c. of the diluted fluid, equal to 2 c.c. of the original saliva. The only other deviation from the conditions already given, consisted in warming the saliva with the alkaline carbonate for 1 hour instead of 30 minutes. We will not give the details of the experiment, as the results were mostly negative. With this amount of saliva, 0·15 per cent. sodium carbonate almost completely destroyed the ferment in 1 hour's warming at 40° C., and even 0·05 per cent. of the alkaline carbonate showed under these conditions a very great destructive action; thus, after heating the diluted saliva with 0·05 per cent. sodium carbonate for 1 hour at 40° C., and then neutralizing the mixture it was able in 30 minutes to convert but 5·69 per cent. of starch into sugar, while the same quantity of saliva simply warmed with water converted under like conditions 27·08 per cent. of starch into sugar.* Under these circumstances, then, the destructive action of dilute sodium carbonate is very great. To what is due this great difference in the action of sodium carbonate of the same strength? Probably to the presence of the larger amount of albuminous matter which in the less diluted saliva possibly combines with the alkaline carbonate. It would follow, moreover, from our results, that any proteid compound formed has in itself no destructive action on the ferment, even to a slight extent. 0·005 per cent. sodium carbonate causes no destruction of the ferment in 1 hour's warming at 40° C.; that is, in saliva of this dilution.

Influence of proteid matter on the diastatic action of saliva in neutral solutions.

It was formerly demonstrated by one of us† that the presence of 1 per cent. of peptone tended to increase the diastatic action of saliva in a neutral solution to such an extent, that on an average about 4 per

* The amount of destruction produced in saliva of this dilution by the above percentage of sodium carbonate does not appear to be constant, since we have found in several cases a much greater diastatic action after an hour's warming at 40° C. than in the above instance, due probably to the larger amount of ptyalin or proteid matter present.

† Chittenden and Ely, Amer. Chem. Jour., vol. iv, 107.

cent. more starch was converted into sugar during 45 minutes at 40° C. ; this with 25 c.c. of saliva in the 100 c.c. of digestive mixture. This effect we attributed to a direct stimulating action on the part of the proteid matter. Langley and Eves,* however, object to this conclusion, although they bring forward no facts to prove the contrary. Considering that litmus will not detect less than 0·001 per cent. of acid or alkali they state that there may be in the neutralized fluid an excess of acid or alkali to this extent, and if, as may well be the case, ptyalin acts best in a neutral solution, the effect of the peptone might be due to its putting *hors de combat* the slight excess of acid or alkali which remains on apparent neutralization. But as Langley himself has shown, the proteid matter naturally present in 25 c.c. of saliva, or even much less, is far more than sufficient to combine with and render inert any such amount of free acid or alkali. We see no other possible explanation of the action of peptones on the diastatic action of saliva in a neutral solution, than a direct stimulation of the ferment. Moreover, Langley and Eves have found that when neutralized saliva is diluted a hundred times, peptone is still able to increase the rate at which it converts starch into sugar, from which they are forced to conclude that the small amount of acid or alkali which may be present, cannot exert, in such a dilution, any retarding influence. We present the following additional results confirmatory of our previous experiments.

In our present experiments we have, however, used much less saliva, and also smaller percentages of peptone.

SERIES XI.

20 c.c. of filtered saliva were neutralized and then diluted to 100 c.c.

0·8 gram of pure albumin-peptone was dissolved in water, made exactly neutral with sodium carbonate and the solution diluted to 100 c.c.

10 c.c. of the diluted saliva were employed in each digestion, and of the peptone solution quantities equivalent to 0·05, 0·1, and 0·2 gram of peptone. Length of digestion, 30 minutes.

Per cent. peptone	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0834 gram.	0·3400 gram.	30·61 per cent.
0·05	0·0875	0·3568	32·11
0·10	0·0868	0·3540	32·01
0·20	0·0873	0·3564	32·04

Here, with the smaller amount of ferment, the increase is not so great as with the larger quantity of saliva and with the longer

* Journal of Physiology, vol. iv, No. 1.

period of digestion; still, the amount of starch converted is increased on an average about 1·50 per cent. It is interesting to note that under these conditions the full effect of the proteid matter is produced by even 0·05 per cent. Langley and Eves found the maximum effect, with saliva ten times diluted, to be produced by about 0·1 per cent. peptone. In our experiment, however, the dilution of the saliva in the digestive mixture is 1 : 50.

Influence of proteid matter on the diastatic action of saliva in alkaline solutions.

It was previously demonstrated by one of us that the presence of 1 per cent. of peptone in a digestive mixture containing 25 per cent. saliva and 0·3 and 0·15 per cent. sodium carbonate respectively tended to nearly double the diastatic action, bringing it up almost to the action of saliva unmixed with alkaline carbonate.

We give here a few additional experiments bearing on this point.

The very noticeable difference in the action of small percentages of sodium carbonate on the diastatic activity of moderately dilute and very dilute saliva, at once suggests the possibility of some connection between the dilution and the reduced percentage of proteid matter. What, now, is the influence of small amounts of peptone on very weak alkaline solutions of saliva? We will give the results of one series of experiments in answer to this question.

SERIES XII.

20 c.c. of saliva with an alkalinity equal to 0·110 per cent. sodium carbonate were diluted to 100 c.c., 10 c.c. of the diluted saliva were used in each digestion of 100 c.c.; consequently the alkalinity of the digestive mixture was equal to 0·0022 per cent. sodium carbonate. Neutral peptone was added in varying quantities. The mixtures were warmed at 40° C. for 30 minutes.

Per cent. peptone.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0761 gram.	0·3104 gram.	27·94 per cent.
0·05	0·0823	0·3352	30·18
0·10	0·0841	0·3424	30·82
0·20	0·0853	0·3480	31·33

The same saliva neutralized, converted 30·61 per cent. of the starch into sugar; consequently the neutral peptone (0·2 per cent.) caused the alkaline saliva to show a diastatic action considerably greater than the neutral saliva, but not equal in this case to the action of the same percentages of peptone on the neutralized saliva. Compare series XI, made with the same saliva.

Still other experiments of the same nature have shown like results, and even more marked. Thus, while neutral saliva without peptone converted in one instance 18·16 per cent. starch into sugar, a like quantity of the normally alkaline saliva (=0·002 per cent. Na_2Co_3 in the digestive mixture) with 0·1 per cent. peptone, converted 31·90 per cent. starch into sugar.

Increasing the percentage of carbonate to a point where previous experiment had shown almost complete stopping of ferment action, it was found that 0·1 per cent. of neutral peptone would, in the above dilution, bring the diastatic action up almost to that of the neutral saliva.

SERIES XIII.

Thus, 20 c.c. of saliva were neutralized and diluted to 100 c.c., 10 c.c. used in each digestion.

	0 Na_2CO_3 0 Peptone.	0·005% Na_2CO_3 0 Peptone.	0·005% Na_2CO_3 0·10% Peptone.
Wt. Cu in one-eighth,	0·0803 gram.	0·0181 gram.	0·0708 gram.
Total amt. sugar,	0·3272	0·0800	0·2896
Starch converted,	29·45 per cent.	7·20 per cent.	26.07 per cent.

With 0·025 and 0·050 per cent. sodium carbonate, 0·1 per cent. peptone availed but little; there was slight diastatic action, but not enough sugar formed to make the determination of any value. These results would seem to indicate that one action of the peptone in an alkaline solution is to combine with the alkaline carbonate and form a compound of quite different power; thus, with 0·050 per cent. sodium carbonate a corresponding larger percentage of peptone is required to increase the diastatic power. In addition to this action, however, there is still manifest the direct stimulating action of the proteid matter on the ferment; seen in one case in the increased percentage of sugar formed in the alkaline solution, over the amount formed in neutral solution by the same saliva under like conditions.

As to the union of peptone and the alkaline carbonate we have a strong indication of a combination of the two, in that the presence of peptone tends to diminish somewhat the destructive action of small percentages of sodium carbonate in diluted saliva.

Thus, while 10 c.c. of neutralized, dilute saliva (1 : 5) warmed for 1 hour with 0·05 per cent. sodium carbonate converted, after neutralization, 25·05 per cent. starch into sugar, the same amount of saliva warmed for the same length of time with the same percentage of sodium carbonate plus 0·4 per cent. peptone converted, after neutralization, 32·68 per cent. of the starch.

The peptone present had evidently in some way prevented the destructive action of the alkaline carbonate, and the most plausible explanation seems to be the probable formation of an alkaline-proteid body.

Influence of free acid and of acid-proteid matter on the diastatic action of saliva.

The influence of dilute acid solutions on the diastatic action of saliva is naturally a point of considerable physiological importance. In view of the rapid passage of the salivary secretions into the stomach, we need to have accurate knowledge of the exact influence of free acid and acid-reacting fluids on the ferment and its diastatic activity.

In considering this question we do not need now to take into account the older observations of Jacubowitsch, Lehmann, Schiff, Watson, Brücke, Hammarsten and others, since these led to no agreement of opinion and more recently acquired knowledge has rendered necessary different methods of procedure.

In 1881 it was announced by one of us* that the ferment of saliva was destroyed on being warmed for two hours with gastric juice containing 0·2 per cent. hydrochloric acid; also that the same treatment with 0·2 per cent. hydrochloric acid alone caused great destruction of the ferment, so that on neutralization diastatic action was greatly diminished. At the same time it was pointed out that much smaller percentages of acid, even 0·025 per cent.,† diminished the diastatic action of the ferment very materially. Shortly after this, similar results were obtained independently by Langley,‡ who in an interesting paper on the destruction of ferments in the alimentary canal, pointed out that ptyalin from the parotids of a rabbit was destroyed by digestion with a small amount of gastric juice, and also that weak solutions of the ferment were more or less destroyed by heating at 40° C. with 0·014 per cent. hydrochloric acid. In comparing these latter experiments with the preceding it is to be remembered that the former were made with 25 c. c. of filtered human saliva, a much stronger solution doubtless, both as regards the ferment and the albuminous matter present.

Later it was pointed out by one of us,§ that peptones have a very

* Chittenden and Griswold, Amer. Chem. Jour., vol. iii, 305.

† Irrespective of the proteid matter.

‡ Journal of Physiology, vol. iii, No. 3.

§ Chittenden and Ely, Amer. Chem. Jour., vol. iv, 114.

decided influence on the diastatic action of saliva in acid solutions; that while the presence of 0·025 per cent. hydrochloric acid prevented the conversion of but 3·50 per cent. of the starch into sugar, the presence of 1 per cent. peptone allowed the conversion of 48·85 per cent. of the starch, 7 per cent. more than the saliva alone would convert under like conditions; a fact which would indicate something more on the part of the proteid matter than a mere union of the peptone and acid. Undoubtedly there was a combination of the peptone and acid, but in addition there was manifested the direct stimulating action of the proteid matter. At the time these experiments were made, however, we were unaware of Danilewsky's* method of testing for free acid with tropæolin 00, by which he proved the union of acids with various forms of proteid matter; compounds acid to test papers, but not containing free acid. Falk† likewise noticed the influence of peptones on diastatic action, in an acid solution of malt infusion; thus by adding a small amount of 0·0135 per cent. hydrochloric acid to an infusion of malt and this to some starch paste, no reaction for sugar could be obtained, but by adding the same proportion of acid and some peptone, then the sugar reaction soon appeared. This fact Falk considered as evidence of the union of the acid and peptone.

In view of these results we have repeated some of our previous work, under different conditions, trying many additional experiments, especially as in a recent paper on the amylolytic action of saliva, Langley and Eves‡ have arrived at some conclusions not in accord with our results.

a. Influence of acid-proteid matter.

We have used the tropæolin test for the detection of free acid, whenever it has been necessary in our work, employing the method as recommended by Danilewsky. The tropæolin 00 was dissolved in methyl alcohol (saturated solution), and when a test for free acid was to be made, drops of the alcoholic solution were allowed to evaporate on a porcelain plate at 40° C., and then while still at 40° C., a drop of the fluid to be tested was added and allowed to dry. Free hydrochloric acid causes the dry residue to take on a violet color. We have made a number of trials to ascertain how small a percentage of free hydrochloric acid can be detected by this test. Using

* Centralbl. Med. Wiss., 1880.

† Virchow's Archivs, lxxxiv, 1881, p. 130.

‡ Loc. cit.

a standard solution of hydrochloric acid of known strength,* we have found that 0·003 per cent. of this acid can be detected with certainty, a drop of such a mixture giving a distinctly recognizable violet color. A smaller percentage cannot be recognized and we have therefore invariably deducted the above amount in our various tests for free acid.

The amount of proteid matter naturally present in saliva and which is capable of combining with acids, is apparently quite constant. Langley and Eves found as a mean of several observations that 5 c.c. of filtered, neutralized saliva contained proteids capable of combining with 2 c.c. of 0·1 per cent. hydrochloric acid. We have found as a mean of eight determinations that 20 c.c. of filtered, neutralized saliva contained proteids capable of combining with 7·74 c.c. 0·1 per cent. hydrochloric acid. In an attempt to ascertain approximately how much proteid matter this amount of acid signified, we took the results of our experiments with peptones, in which we found that 1 gram of pure neutral peptone required 48·0 c.c. 0·1 per cent. hydrochloric acid to saturate it. Consequently 1 c.c. of 0·1 per cent. acid would combine with 0·0208 gram peptone, and assuming that the combining power of the proteids present in saliva is the same as that of peptones, the 20 c.c. of saliva would contain 0·16099 gram proteid matter, equal to 0·804 per cent.; a result which at once shows that the combining power of the proteids of saliva and peptone must be quite different, or as is more probable, that considerable of the acid added, is used up in reacting with the phosphates of the alkalies present in the saliva.

Saliva, as a rule, does not contain much more than 0·5 per cent. solid matter, and Hammerbacher has found in human mixed saliva 0·139 per cent. albumin and ptyalin.†

A comparison of the diastatic action of neutral saliva considerably diluted, and similarly diluted saliva in which the proteids present have been saturated with acid, shows at once that acid-proteid matter, even though present in but small quantity, has a distinctly stimulating action on the salivary ferment.

The following experiments will illustrate this point and also show the extent of the stimulation.

SERIES XIV.

A. 40 c.c. filtered saliva were neutralized and then diluted to 200 c.c.

* All of our standard acid solutions were of exactly the strength specified, as was proved by titration with standard solution of silver nitrate.

† Jahresbericht für Thierchemie, 1881, p. 269.

B. 40 c.c. of the above diluted saliva required 6·8 c.c. 0·05 per cent. HCl to saturate the proteids present = 0·0074 per cent. combined HCl.

Two digestions each were made with *A* and *B*, using quantities of the above salivas equivalent to 4 and 2 c.c. of the original saliva.

	Wt. Cu in one-eighth.	Total amt. sugar formed.
20 c.c. *A*,	0·0913 gram.	0·3720 gram.
23·4 *B*,	0·0987	0·4016
10 c.c. *A*,	0·0850 gram.	0·3472 gram.
11·7 *B*,	0·0940	0·3832

	Starch converted.		Starch converted.
20 c.c. *A*,	33·49 per cent.	10 c.c. *A*,	31·26 per cent.
23·4 *B*,	36·15	11·7 *B*,	34·49
Increase,	2·66 per cent.	Increase,	3·23 per cent.

It is seen that addition of the acid in this instance causes a very decided increase in the diastatic activity of the saliva. The amount of combined acid present in the 100 c. c. of digestive mixture in the two cases was 0·0017 and 0·0008 per cent. respectively, yet the presence of this small amount of combined acid manifestly acts as a stimulant to the diastatic ferment.* Even still smaller percentages of acid-proteid matter have an equally decided action on the salivary ptyalin. The following series of experiments illustrate this point and at the same time are confirmatory of the preceding one.

SERIES XV.

A. 40 c.c. filtered saliva were neutralized and diluted to 200 c.c.

B. 50 c.c. of the above diluted saliva required 4·75 c.c. 0·05 per cent. HCl to saturate the proteids. The solution was distinctly acid to litmus paper and contained 0·0043 per cent. combined HCl.

Four digestions were made with both *A* and *B*, using quantities of saliva in each case equivalent to 4, 2, 1 and 0·5 c.c. of the original saliva.

	Wt. Cu in one-eighth.	Total amt. sugar formed.
20 c.c. *A*,	0·0925 gram.	0·3768 gram.
21·9 *B*,	0·0959	0·3912
10 c.c. *A*,	0·0827 gram.	0·3368 gram.
10·95 *B*,	0·0876	0·3576
5 c.c. *A*,	0·0671 gram.	0·2744 gram.
5·5 *B*,	0·0751	0·3064
2·5 c.c. *A*,	0·0305 gram.	0·1296 gram.
2·75 *B*,	0·0375	0·1568

* Doubtless these percentages of combined acid are too high, since as before mentioned some of the acid added probably reacts with the phosphates naturally present in the saliva.

	Starch converted.		Starch converted.
20 c.c. *A*,	33·85 per cent.	10 c.c *A*,	30·32 per cent.
21·9 *B*,	35·22	10·95 *B*,	32·19
Increase,	1·37 per cent.	Increase,	1·87 per cent.
5 c.c. *A*,	24·69 per cent.	2·5 c.c. *A*,	11·68 per cent.
5·5 *B*,	27·58	2·75 *B*,	14·10
Increase,	2·89 per cent.	Increase,	2·42 per cent.

Here the same results are to be seen as in the preceding experiment, although the amount of proteid matter is much less. In both series of experiments it is to be noticed that as the percentage of combined acid is diminished, the difference between the diastatic activity of the neutral solution and the corresponding acid solution is increased; at the same time it is to be seen that in the first series of experiments where the percentage of proteid matter is larger, there is a greater increase in the conversion of starch with the 23·4 c.c. of acid-reacting saliva than with the 21·9 c.c. of the acid-reacting fluid of the second series of experiments, with its smaller percentage of proteid matter.

In the last series of experiments where 21·9 c.c. of *B* are used, the amount of combined acid in the digestive mixture is but 0·00094 per cent. HCl, so that where the smaller amounts of acid-reacting saliva are used the percentage amount of combined acid is very small indeed.

Increasing the amount of saliva used and thereby the percentage of acid-proteid matter, brought us finally to a point where the acid-proteid matter failed to stimulate the diastatic action of the ferment and even began to show a tendency to retard its action. The following series of experiments, using saliva wholly undiluted, illustrates this point.

SERIES XVI.

100 c.c. of filtered saliva were neutralized, requiring 32 c.c. 0·2 per cent. HCl = *A*.

52·8 c.c. *A* = 40 c.c. of the original saliva required 12·15 c.c. 0·1 per cent. HCl to combine with the proteids, making saliva *B*; the fluid was distinctly acid to litmus and contained 0·0187 per cent. combined acid. Three digestions were made with both *A* and *B*, using quantities of the fluids equal to 20, 10 and 5 c.c. respectively of the original saliva.

	Wt. Cu in one-eighth.	Total amt. sugar.
26·4 c.c. *A*,	0·1083 gram.	0·4408 gram.
32·48 *B*,	0·1065	0·4336
13·2 c.c. *A*,	0·1024 gram.	0·4168 gram.
16·24 *B*,	0·1087	0·4424
6·6 c.c. *A*,	0·0948 gram.	0·3864 gram.
8·12 *B*,	0·1031	0·4192

	Starch converted.	Combined HCl in the 100 c.c. digestive mixture.
26·4 c.c. *A*,	39·68 per cent.	0
32·48 *B*,	38·96	0·00608 per cent.
Decrease,	0·72 per cent.	
13·2 c.c. *A*,	37·52 per cent.	0
16·24 *B*,	39·73	0·00304 per cent.
Increase,	2·21 per cent.	
6·6 c.c. *A*,	34·79 per cent.	0
8·12 *B*,	37·74	0·00152 per cent.
Increase,	2·95 per cent.	

In this series of experiments, where the percentage of combined acid in the digestive mixture is much greater than before, the same increase in diastatic action is noticed. With the largest quantity of saliva, however, where the amount of combined acid is 0·006 per cent. we seem to have reached a point where the acid-proteid matter ceases to stimulate and begins to retard the action of the ferment. That this is actually the case we have proved by another experiment confirmatory of the preceding one, using in the digestion however, two grams of starch instead of one.

Thus while an amount of neutral saliva, equal to 20 c.c. of the original secretion converted 39·08 per cent. starch into sugar, the same amount of saliva, having all of its proteid matter combined with acid, converted under the same conditions 38·21 per cent. of the starch, a decrease of 0·87 per cent.; in this case, however, the amount of combined acid present in the 100 c.c. of digestive mixture was 0·008 per cent.

It thus seems plainly proven that up to a certain percentage, the presence of acid-proteid matter in the saliva tends to decidedly stimulate its diastatic action. We cannot therefore agree with Langley and Eves that ptyalin acts best in every instance in a neutral solution, for our results certainly show an increased action of the ferment in the presence of acid-proteids, except where the latter are present in comparatively large amount.

The only possible fallacies which suggest themselves here are traces of undetectable alkali in the starch and the presence of phosphates of calcium or magnesium. This result moreover, makes clear many statements previously recorded which would otherwise be difficult of explanation. Thus it has been recorded by Astaschewsky,* that the saliva of the parotid gland possesses a very faint acid reac-

* Centralbl. med. Wiss., 1875, 15.

tion and that the maximum diastatic action of parotid saliva corresponds with the strongest acid reaction; but in these observations doubtless the acid reaction was in every case due to acid-proteids and not to free acid. Again, it was found by one of us* that the presence of 0·005 per cent. HCl decidedly increased the diastatic action of saliva, but while the observation was correct the result was wrongfully attributed to 0·005 per cent. free acid when it should have been attributed to the same percentage of combined acid, where doubtless the proteid matter was not wholly saturated. Likewise Watson's† oft-quoted result, where the addition of a drop of strong acid to saliva gave him an increased diastatic action, was doubtless due to the acid-proteid matter formed and not to free acid, though it may have been due to partial or complete neutralization.

We endeavored to ascertain whether the acid-proteid matter formed by the addition of acid to undiluted saliva would have any destructive action on the diastatic ferment when warmed at 40° C. Of course only a slight action, if any, could be expected, still it seemed of sufficient importance to warrant the experiment. Accordingly two mixtures were prepared as follows :

	A.	*B.*
Saliva,	20 c.c.	20 c.c.
HCl 0·2% to neutralize,	6·8	6·8
" " combine with proteids,		3·2
H_2O,	13·2	10
	40·0 c.c.	40·0 c.c.
	Neutral.	0·016% HCl combined.

These two solutions were warmed at 40° C. for 1 hour, then neutralizing and equalizing‡ mixtures were added, after which starch and water to 100 c.c. The results were in *A* a conversion of 38·68 per cent. of the starch into sugar, and in *B* a conversion of 38·26 per cent., so that while there may have been some little destruction of the ferment, it is plain that the diminished action noticed in the two preceding cases in the presence of the larger percentages of acid-proteid matter was probably due to simple retardation, since the percentage of combined acid was not more than half that in the above experiment.

We have studied the influence of acid-proteid matter on salivary

* Chittenden and Griswold, Amer. Chem. Jour., vol. iii, 312.

† Jour. Chem. Soc., 1879, 543.

‡ Equivalent amounts of standard acid and sodium carbonate solutions, so that *A* for example might contain the same amount of sodium chloride as *B*.

digestion still further by experimenting likewise with peptones, and in studying the influence of acid-peptones on the action of the ferment we have been impressed with the striking effect of very minute quantities of acid on the ordinary action of peptones, increasing it very decidedly. It has already been shown that the presence of 0·05, 0·1 and 0·2 per cent. of neutral peptone produces in neutral solutions, a like increased diastatic action; with 0·5 per cent. peptone the increase is as much as with 0·2 per cent.; that is, in the case of saliva considerably diluted. With acid-peptones, however, the effect produced is quite different, and the amount of combined acid necessary to produce this different effect is quite small.

Peptones as usually prepared contain a small amount of combined acid. The sample we used required per gram 0·014 gram Na_2Co_3 to make it neutral; this would be equivalent to 0·00964 gram HCl. Consequently the percentage of combined acid in the peptone, assuming it to be hydrochloric acid, would be 0·964 per cent. With such an acid-peptone the following experiments were tried.

SERIES XVII.

20 c.c. saliva were neutralized and diluted to 100 c.c.; of this solution 10 c.c., equal to 2 c.c. of original saliva were used in each digestion. Four experiments were tried, in three of which 0·050 gram, 0·100 gram and 0·200 gram of the above acid-peptone were added. Following are the results, after warming the mixtures at 40° C. for 30 minutes.

Per cent. peptone.	Wt. Cu in one-eighth.	Total amt. sugar formed.	Starch converted.
0	0·0766 gram.	0·3128 gram.	28·16 per cent.
0·05	0·0873	0·3560	32·05
0·10	0·0897	0·3656	32·91
0·20	0·0929	0·3784	34·21

Comparing these results with those obtained by similar percentages of neutral peptone the difference is sufficiently striking, and yet the percentage of combined acid in the digestive mixture, where there is present 0·20 gram of acid peptone, is but 0·0019 per cent. calculated as HCl.

Experimenting with peptones completely saturated with acid, and in this case with what was known to be hydrochloric acid, results similar to the above were obtained, with, however, several suggestive deviations. The following series of experiments will serve to illustrate the main points of interest.

SERIES XVIII.

40 c.c. filtered saliva were neutralized and diluted to 200 c.c.; 10 c.c. of this diluted fluid were used in each experiment.

A standard solution of peptone saturated with hydrochloric acid was also prepared.

The following percentages of peptone and combined acid were contained in the different digestive mixtures of 100 c.c.

	1	2	3	4	5	6	7
Peptone,	0	0	0·01%	0·020%	0·040%	0·060%	0·080%
Combined HCl,	0	0·0006%*	0·00057%	0·00115%	0·0023%	0·00345%	0·0046%

Following are the results of the digestions:

	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
No. 1	0·0872 gram.	0·3560 gram.	31·85 per cent.
2	0·0896	0·3656	32·91
3	0·0901	0·3672	33·06
4	0·0935	0·3808	34·28
5	0·0892	0.3640	32·77
6	0·0775	0·3160	28·45
7	0·0495	0·2048	18·43

It is to be noticed, first, that in this series of experiments the peptones, being completely saturated with acid, are present in much smaller percentages proportionally than the combined acid is, and the effect produced is a diminished diastatic action in the case of Nos. 6 and 7, in the presence of an amount of combined acid which in the case of the proteids naturally present in saliva has no retarding action whatever, but on the contrary a stimulating action. The addition of a larger amount of peptone to Nos. 5, 6 and 7, for example, the percentage of acid remaining the same, tends to bring up the diastatic action very decidedly.

It would appear from these results, moreover, assuming that the combining power of peptone is the same as the proteids present in saliva, that the presence of say 0·003 per cent. combined HCl in the form of saturated acid-peptone has a retarding action, while the same percentage of combined HCl in the form of saturated salivary proteids, has, in the case of saliva of the same dilution, a decided stimulating action. At the same time it is to be remembered that when acid is added to saliva some considerable acid may be used by the inorganic salts with formation of acid phosphates, for example. These results, moreover, indicate that such is doubtless the case. Increasing the percentage of peptone to say 1 per cent. admits of the addition of larger amounts of hydrochloric acid, without partic-

* To saturate the proteids naturally present in the saliva.

ularly retarding the action of the ferment; thus, as Langley and Eves state, "0·0075 per cent. hydrochloric acid may be added to saliva diluted 10 or 20 times, provided 1 per cent. peptone be present, and yet its action on starch be about equal to that of the saliva without peptone or acid."

Again it would appear that small percentages of acid-proteid matter, either peptones or the albuminous bodies present in saliva, tend to increase the diastatic action not only beyond the natural action of the saliva, but also somewhat beyond the action of the saliva plus the same percentage of neutral peptone. Thus, while the presence of a few hundredths of one per cent. of neutral peptone in saliva diluted 1:50 caused about 1·5 per cent. increased conversion of starch, acid-peptone caused in 30 minutes 2·17 per cent. increased conversion. Again, as has been seen, the acid-proteids of saliva cause a like increase. Large percentages of acid-proteids, however, in which the albuminous matter is completely saturated, distinctly retard diastatic action.

These results harmonize in a general way with the previous results obtained by one of us,* in which it was found that the presence of 1 per cent. peptone in an acid reacting fluid, which by itself almost completely stopped the diastatic action of the saliva, increased the diastatic action of the ferment above the action of the neutral saliva and also above the action of the neutral saliva plus the 1 per cent. of neutral peptone.

We next endeavored to ascertain how much of the retarding action of acid-peptone is due to destruction of the ferment. Without giving details we have found that with saliva ten times diluted there is a noticeable destruction of the ferment in the presence of 0·028 per cent. of combined acid, although it is not great. In this case it is to be understood that the amount of peptone present is only such as would furnish this percentage of combined acid. The following percentages of starch converted (after neutralization and equalization) show the amount of destruction under the different conditions.

SERIES XIX.

Length of time at 40° C.	Per cent. combined HCl.	Per cent. peptone.	Starch converted.
	0	0	31·65 per cent.
30 min.	0·014	0·25	31·18
30	0·028	0·50	30·82
60	0·028	0·50	29·74
30	0·057	1·00	27·73

* Chittenden and Ely, loc. cit.

It is thus manifest from our results that the retarding influence of the larger percentages of acid-peptones is out of all proportion to their power of destruction. Still larger percentages of acid-saturated peptones produce a much greater destruction. Thus, by warming 10 c.c. of a neutral dilute saliva (1 : 5) with a solution of peptone saturated with acid, in such proportion that the mixture contained 0·430 per cent. combined HCl, there was in 30 minutes an almost complete destruction of the ferment.

b. Influence of free acid.

In view of the fact that some time ago one of us* was of the opinion that small percentages of acid† tended to increase the diastatic action of saliva, it was of especial interest now to ascertain definitely whether free acid when present in small percentages does invariably retard diastatic action. Langley and Eves state that "although saliva neutralized to litmus sometimes shows an increase of action on the addition of 0·0005 to 0·001 per cent. HCl; yet if the proteids of the saliva be saturated with acid, there is a diminution of its amylolytic action, although no free acid is present in the saliva." This we cannot regard as correct without qualification, since our experiments appear to show that saliva with its proteid matter saturated with acid has a greater diastatic action in a given time than saliva simply neutralized, provided the percentage of acid-saturated proteids is not too large. The same investigators further state "that 0·0015 per cent. HCl distinctly diminishes the amylolytic action of pytalin," and "since 0·0015 per cent. HCl decreases amylolytic action it seems very unlikely that 0 0005 per cent. should increase it;" but as Langley and Eves, in studying the influence of free acid, apparently used diluted, neutralized saliva, in which the proteids present were not combined with acid, depending simply upon dilution to avoid the influence of these bodies, it seems to us a little uncertain whether their results are strictly accurate on this point, since saliva even very much diluted does contain some proteid matter. They, however, state in this connection that "we have often found that solutions which we have thought carefully neutralized have been increased in action by the presence of still smaller percentages of acid, viz: 0·0005 to 0·0010 per cent." Here, however, so far as their results show, the observed increase of activity may have been due to

* Chittenden and Griswold, loc. cit.

† Considered as 0·005 per cent., although we now know the above figure could not represent free acid, owing to the proteid matter of the saliva.

the small amount of acid-proteid matter present, certainly could not have been due wholly to free acid.

We have tried a large number of experiments on this point in a variety of ways, all of which tend to show that a very small trace of free acid, when the amount of acid-proteids is not large, does, seemingly, slightly increase the diastatic action of the ferment. It is perhaps, questionable, whether in the use of such small percentages of acid, the results are to be strictly depended upon. The presence of a small amount of phosphate in the starch or a trace of alkali, not to be detected by litmus, might easily neutralize the small amount of acid added. Again, non-saturation of the proteids to only a very slight extent might affect the result. We subjoin two or three of our experiments.

SERIES XX.

20 c.c. filtered saliva were neutralized and then sufficient acid added to combine with the proteids present; the mixture then diluted to 100 c.c. The solution contained 0·0114 gram combined HCl, but no free acid. *A*.

20 c.c. of the same filtered saliva neutralized, and the proteids just saturated with acid. 3·1 c.c. 0·1 per cent. HCl were then added and the mixture diluted to 100 c.c. The solution contained 0·0114 gram. combined HCl, and in addition 0·0031 gram free HCl. The solution gave a distinct violet with tropæolin 00. *B*.

Digestions were made, using 1 gram of starch in a volume of 100 c.c. Time, 30 minutes. Following are the results.

Amount diluted saliva.	*A*. Wt. Cu in one-eighth.	*A*. Total amt. sugar.	*B*. Wt. Cu in one-eighth.	*B*. Total amt. sugar.
20 c.c.	0·0988 gram.	0·4024 gram.	0·0972 gram.	0·3960 gram.
10	0·0917	0·3736	0·0921	0·3752
5	0·0826	0·3368	0·0861	0·3512

Amount diluted saliva.	Starch converted. *A*.	Starch converted. *B*.	Free HCl in *B*.
20 c.c.	36·23 per cent.	35·65 per cent.	0·00062 per cent.
10	33·53	33·78	0·00031
5	30·32	31·62	0·00015

Here there can be no question but that there was free acid in *B*. The saliva gave a distinct reaction with tropæolin 00 and the starch used was apparently neutral. In this instance 0·0006 per cent. free acid slightly diminished diastatic action, while 0·0003 per cent. slightly increased it.

A second experiment of like nature gave the following results:

SERIES XXI.

20 c.c. filtered saliva were neutralized and the proteids exactly saturated with HCl, then diluted to 100 c.c. The solution contained 0·0073 per cent. combined HCl, but no free acid. *A.*

20 c.c. of the same saliva neutralized and the proteids saturated by the addition of the same amount of acid as in *A*; 1·2 c.c. 0·1 per cent. HCl were then added, so that a distinct tropæolin reaction could be obtained in the 41 c.c. of fluid. The fluid was diluted to 100 c.c. and then contained 0·0012 per cent. free HCl. *B.*

20 c.c. of the same saliva, neutralized and the proteids exactly saturated with acid; then enough more acid added to give a distinct tropæolin reaction, after which the solution was diluted to 100 c.c. The 100 c.c. of fluid contained exactly 0·003 gram HCl. *C.*

A drop of the latter fluid on being tested gave a distinct violet with tropæolin 00.

Following are the results of digestions made with the foregoing solutions of saliva.

Amount of diluted saliva.	Starch converted.		
	A.	*B.*	*C.*
20 c.c.	35·65 per cent.	35·58 per cent.	35·36 per cent.
10	33·71	33·27	34·14
5	28·81	29·53	30·32

Here it is seen, as before, that the smaller percentages of free acid arising from the use of 5 and 10 c.c. of saliva, show a distinctly increased diastatic activity, while with 20 c.c. the results are very nearly identical; too large an amount of free acid to increase the action and yet not enough to materially diminish it.

We next tried the influence of *increased* percentages of free acid.

SERIES XXII.

30 c.c. filtered saliva were neutralized and the proteids just saturated with acid, then diluted to 150 c.c.; 10 c.c. of this diluted saliva equal to 2 c.c. of the original saliva were used in each digestion. Following are the results, after warming with starch at 40° C. for 30 minutes in the presence of the percentages of free acid specified. The acid solutions were mixed with the starch previous to the addi tion of the saliva.

Per cent. free acid.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0919 gram.	0·3744 gram.	33·71 per cent.
0·0006	0·0924	0·3768	33·92
0·0010	0·0773	0·3152	28·37
0·0020	0·0166	0·0744	6·69
0·0030	trace		

Here a slight increase is noticed with 0·0006 per cent. followed at 0·002 per cent. by a rapid fall in diastatic action.

With stronger solutions of ptyalin, like results were obtained as follows:

SERIES XXIII.

Filtered saliva was neutralized and the proteids just saturated with HCl. An amount of this fluid equivalent to 5 c.c. of the original saliva was used in each digestion. In this amount there was present 0·00266 gram combined HCl, but no free acid whatever.

Following are the results of digestions with this saliva in the presence of the percentages of free acid specified.

Per cent. free acid.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0956 gram.	0·3896 gram.	35·07 per cent.
0·0005	0·0966	0·3936	35·43
0·0010	0·0867	0·3536	31·80
0·0020	0·0162	0·0728	6·55
0·0030	trace		

Increasing now the amount of saliva still further, so that the percentage of combined acid reaches a point where its retarding influence begins to be felt, the presence of the smallest amount of free acid then causes at once a decided decrease in diastatic action. Thus, using the same saliva as was employed in the preceding series, only in such quantity that 20 c.c. of original saliva were present in each digestive mixture, it was found that the free acid produced a much greater retarding effect than before. The percentage of combined hydrochloric acid, in the form of acid-proteids, contained in each digestive mixture was 0·01064 per cent. Following are the results of diastatic action.

Per cent. free acid.	Wt. Cu in one-eighth.	Total amt. sugar.	Starch converted.
0	0·0972 gram.	0·3960 gram.	35·65 per cent.
0·0005	0·0830	0·3384	30·46
0·0010	0·0410	0·1712	15·41
0 0020	0·0061	0·0328	2·95
0·0030	trace		

This result accords with the statement made by Langley and Eves, "that if the proteids of saliva be saturated with acid there is a diminution of its amylolytic action, although no free acid is present in the saliva. This diminution is made more marked by the addition of the smallest quantity of hydrochloric acid." The above quantitative results plainly testify to the accuracy of the latter part of their statement. As to the action of the acid-saturated proteids, that is wholly dependent upon the percentage present.

c. Destructive action of free acid.

It has been clearly shown* that acid approximating to the strength of the acid of the gastric juice has a destructive action on the salivary ferment. Smaller percentages of acid have a like destructive action. It has at the same time been shown that the presence of very much smaller percentages of free acid stops the amylolytic action of the ferment. Is this stopping of amylolytic action in every case due to destruction of the ferment, or simply to the retarding action of its presence? Langley, by using an aqueous extract of the parotid of rabbits, with but little proteid matter, concluded that the presence of 0·014 per cent. hydrochloric acid is sufficient to destroy all but the merest trace of ferment in five minutes at 39° C. This before the action of acid-proteids was known. Chittenden and Ely by experimenting with human saliva came to the conclusion "that there may be in the presence of a *very dilute* acid, a simple stopping of diastatic action, without destruction of the ferment;" in other words, the retarding influence of very small percentages of free acid is not necessarily due to destruction of the ferment. Langley and Eves criticising this conclusion state "that since Chittenden and Ely apparently used unneutralized saliva and took no account of the proteids present, it seems to us probable that not only was there no free hydrochloric acid in their experiments, but that even the proteids were not saturated with acid." In the article to which they refer it is, however, explicitly stated in a foot note† that the saliva was neutralized and then an amount of acid added to equal 0·025 per cent. Unfortunately, we did not then know of the action of acid on the proteids of the saliva; consequently, the above percentage must have been mainly in the form of combined acid. Still, the smaller percentages of free acid do not show great destructive action; their power of retarding the action of the ferment is out of all proportion to their power of destruction. Amylolytic action is almost entirely stopped by the presence of 0·002 per cent. free hydrochloric acid, but warming saliva at 40° C. with 0·002 or even 0·005 per cent. hydrochloric acid for 30 minutes causes little if any destruction of the ferment. On neutralization, diastatic action goes on as vigorously as ever.

This is well illustrated by the following experiments:

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* Chittenden and Griswold, loc. cit.; Chittenden and Ely, loc. cit.; Langley, loc. cit

† Amer. Chem. Jour., vol. iv, p. 119.

SERIES XXIV.

20 c.c. of filtered saliva were neutralized, the proteids just saturated with acid and the mixture diluted to 100 c.c. The solution contained 0·007 per cent. combined HCl.

10 c.c. of this diluted saliva were warmed with the specified percentages of acid for a definite time, then neutralizing and equalizing mixtures were added and diastatic action determined.

Following are the results.

Length of time at 40° C.	Per cent. of free HCl.	Starch converted.
30 minutes,	0	32·63 per cent.
30	0·001	34·08
30	0·002	31·38
60	0·002	32·48
30	0·005	31·27
30	0·010	4·60
30	0·030	Complete destruction.

Although the results are for some reason a little irregular it is very evident that up to 0·005 per cent. of free acid there is, under these conditions, no particular destruction of the ferment. With 0·010 per cent. on the other hand destruction is very great.

As to the bearing which these results have on the possible amylolytic action of saliva in the stomach, it is plain that when the fluids of the stomach acquire an acid reaction due to the presence of free hydrochloric acid, ptyalin will soon be destroyed. In the first stage of digestion, however, when there is no free acid, the conversion of starch into sugar can undoubtedly go on, and at this stage of the process the proteid matter present may act as a shield to protect the ptyalin and at the same time to stimulate it in its action; but as the acid-proteids increase in amount and come nearer and nearer to their saturation point it is possible that diastatic action may entirely stop, even before free acid makes its appearance. Certainly all salivary ptyalin must ultimately be destroyed in the stomach.

General conclusions.

1. The diastatic action of saliva can be taken as a definite measure of the amount of ferment present, only when the dilution of the saliva in the digestive mixture is as 1 : 50 or 100. The limit of dilution at which decisive diastatic action will manifest itself with formation of reducing bodies is 1 : 2000–3000, under the conditions previously given.

2. The diastatic action of neutralized saliva is greater than that of normally alkaline saliva. The difference is particularly noticeable

where the dilution is as 1:50 or 100, and is apparently out of all proportion to the amount of alkalinity.

3. Sodium carbonate retards the diastatic action of ptyalin in proportion to the amount of alkaline carbonate present. The percentage of alkaline carbonate, however, which hinders diastatic action can be designated only for definite mixtures and not in a general sense, being dependent upon the dilution of the saliva and the consequent change in percentage of proteid matter.

4. The destructive action of sodium carbonate is modified materially by the dilution of the saliva; becoming greater the more the fluid is diluted. This result is due not to simple dilution but doubtless to the diminished amount of proteids.

5. Neutral peptone has a direct stimulating effect on the diastatic action of neutral saliva.

6. The presence of small percentages of neutral peptone tends to raise the diastatic action of normally alkaline saliva, to a point even beyond the action of the neutralized fluid; due in part doubtless to a loose combination of the alkali with the proteid matter, and also to a direct stimulation of the ferment. Likewise, peptone tends to diminish in a similar manner the retarding action of the various percentages of sodium carbonate. To accomplish this, however, the amount of peptone must be proportionate to the percentage of alkaline carbonate.

7. Peptone tends to prevent the destructive action of dilute sodium carbonate on salivary ptyalin, thus giving proof of the probable formation of an alkaline-proteid body.

8. Saliva with its proteid matter saturated with acid appears to have a greater diastatic action than when simply neutralized; except when the acid-proteids thus formed are above a certain percentage. Small percentages of peptone saturated with acid, similarly increase the diastatic action of neutralized saliva up to a certain point. Increasing the percentage of acid-proteids finally causes a diminution of diastatic activity.

9. The retarding influence of acid proteids is out of all proportion to their power of destruction. Large percentages, however, of acid-proteids may cause almost complete destruction of the ferment.

10. The most favorable condition for the diastatic action of ptyalin, under most circumstances, appears to be a neutral condition of the fluid together with the presence of more or less proteid matter. The addition of very small amounts of hydrochloric acid, however, to *dilute* solutions of saliva, giving thereby a *small percentage* of acid-

proteids, appears to still further increase diastatic action. Under *such conditions* a minute trace of free acid appears to still further increase the action.

11. 0·003 per cent. free hydrochloric acid almost completely stops the amylolytic action of ptyalin. The larger the amount of saturated proteids the more pronounced becomes the retarding action of free acids.

12. The retarding effects of the smaller percentages of free acid are not due wholly to destruction of the ferment. Pronounced destruction takes place with 0·005–0·010 per cent. free hydrochloric acid.

13. Proteid matter, in influencing the diastatic activity of salivary ptyalin acts not only by combining with acids and alkalies, but apparently also by direct stimulation of the ferment.

[From the Transactions of the Connecticut Academy, Vol. VII, 1885.]

The Amylolytic action of Diastase of Malt, as modified by various conditions; studied quantitatively.

By R. H. Chittenden and Geo. W. Cummins, Ph.B.

The close relationship existing between the diastase of malt and the amylolytic ferment of saliva has led us to make a careful study of the conditions favorable to the action of the former, in the hope of obtaining confirmation of previous results obtained with the salivary ferment.* The widespread use, moreover, of malt extracts as therapeutic agents lends to the work in question a practical interest, which in no wise detracts from its value.

Falk† has recorded that the diastase of malt loses its amylolytic power under the influence of dilute acid, similar to the ferment of saliva; that it is made inactive by gastric juice and that the retarding influence of a dilute acid (say 0·0135 per cent.) on its amylolytic power is diminished by the presence of peptone, owing to the probable formation of a peptone-acid compound. Falk, moreover, states that the retarding action of hydrochloric acid is due to destruction of the ferment, since on neutralization of the acid, amylolytic power is not restored. Kjeldahl‡ has recorded that dilute acids in very small quantity retard the amylolytic action of diastase; if, however, smaller, minimum quantities of acid are added the amylolytic power of diastase is increased. The same investigator§ has also noticed a like accelerating action of very small quantities of acid on invertin. Basnitz‖ has found that the presence of carbonic acid invariably increases the amylolytic power of diastase. Detmer¶ has recorded the same fact and in addition, that small quantities of citric acid as well as of phosphoric and hydrochloric acid increase the diastatic power of malt. Larger quantities of these acids render the malt extract inactive. Detmer has also found that the presence of a very slight alkaline reaction diminishes the amylolytic power of the ferment. Brown and Heron** state that a malt extract neutralized with barium hydroxide has its amylolytic power somewhat weakened; thus im-

* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

† Virchow's Archiv, vol. lxxxiv, p. 119.

‡ Jahresbericht für Thierchemie, 1879, p. 382.

§ Jahresbericht für Thierchemie, 1881, p. 449.

‖ Berichte der deutsch chem. Gesell., vol. xi, p. 1443.

¶ Zeitschrift für physiol. chemie, vol. vii, p. 2.

** Liebig's Annalen der Chemie, vol. cxcix, pp. 236–238.

plying that the ferment acts more vigorously in the naturally acid extract than in a neutral fluid. The same investigators found that making the extract faintly alkaline with sodium carbonate also diminished somewhat the activity of the ferment, while sodium hydroxide completely stopped the action of the ferment. A like result was also obtained on the addition of 0·05 per cent. salicylic acid.

Such are the recorded statements bearing on this question. Few quantitative results are given, and the influence of proteid matter, aside from its connection with dilute acid, has not been considered.

Method employed.

A fresh malt extract was prepared for each series of experiments, since the fluid tends rapidly to become acid, owing to the development of schizomycetes. The extract was prepared from coarsely ground malted barley, by simply extracting it with water at 40° C. for two to three hours (5 grams barley to 100 c. c. water), then filtering, neutralizing and diluting to 500 c. c.

Owing to the great difficulty of obtaining perfectly neutral starch, that used in the present work was prepared from potatoes, thoroughly washed and dried, making a starch perfectly neutral to the most delicate test papers. The volume of each digestive mixture in the various experiments was 100 c. c., containing 1 gram of starch previously boiled with a portion of the water, a definite quantity of the malt extract and a given amount of acid, alkali, or proteid matter, except in the control, which was naturally free from the latter. In determining amylolytic power the digestive mixtures were warmed at 40° C. for thirty minutes, after which further ferment action was stopped by boiling the fluid. The extent of amylolytic action was then ascertained by determining in one-fourth of the fluid, made up to 100 c. c., the amount of reducing bodies by means of Allihn's* gravimetric method; the reducing bodies being then calculated, for the sake of convenience, to dextrose, from which in turn, was calculated the percentage of starch converted.

Influence of sodium carbonate on the amylolytic action of diastase.

Previous experiments† with saliva have shown that the percentage of alkaline carbonate which absolutely or to a certain extent hinders its amylolytic action can be designated only for a definite mixture and not in a general sense, owing to variations in the amount of pro-

* Zeitschrift für Analytische Chemie, vol. xxii, p. 448. † Chittenden and Smith.

teid matter present and doubtless also in part to increase or decrease in the amount of ferment.

It became necessary, therefore, at first, to ascertain something regarding the relative amylolytic action of the malt extract, which contains some proteid matter. Three quantities of malt extract were employed, which by thirty minutes warming at 40° C. with 1 gram of starch in the manner already described, gave the following results:

Malt extract.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
10 c. c.	0·1192 gram.	0·2428 gram.	21·85 per cent.
15	0·1489	0·3038	27·34
25	0·1543	0·3150	28·35

It is interesting to note here that, as in the case of the amylolytic ferment of saliva, there is no quantitative relation between the amount of ferment and the extent of amylolytic action; it is only when the ferment solution is greatly diluted that amylolytic action can be taken as a definite measure of the amount of ferment present. Kjeldahl* has likewise studied the influence of the quantity of diastase upon the amount of sugar formed under given conditions and he came to the conclusion that the formation of sugar was proportional to the amount of ferment only up to a certain point; beyond which, increase in the amount of ferment was not accompanied by proportional increase in the formation of sugar.

Preliminary experiments showed us that the ferment of malt is very susceptible to the action of sodium carbonate; the addition of even 0·025 gram of the alkaline carbonate to 15 c. c. of perfectly neutral malt extract, with subsequent dilution to 100 c. c. allowed no diastatic action whatever. Following are two series of experiments illustrating the action of different percentages of sodium carbonate† on the ferment under different degrees of dilution.

a. with 15 c. c. of the standard malt extract.

Na_2Co_3.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1371 gram.	0·2794 gram.	25.14 per cent.
0·0005 per cent.	0·1318	0·2684	24·15
0·0010	0·1274	0·2596	23.36
0·0020	0·0544	0·1124	10·11
0·0050	0·0197	0·0434	3·90
0·0080	0·0134	0·0312	2·80
0·0100	0·0135	0·0314	2·82
0·0125	0·0065	0·0148	1·33
0·0250	0		

* Jahresbericht für Thierchemie, 1879, 381.

† The standard solutions of sodium carbonate employed in these experiments were made from the chemically pure anhydrous salt.

b. with 30 c. c. of the standard malt extract.

Na_2Co_3.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1650 gram.	0·3372 gram.	30·34 per cent.
0·001 per cent.	0·1578	0·3224	29·01
0·003	0·1465	0·2986	26·87
0·005	0·1147	0·2334	21·00
0·010	0·0380	0·0796	7·16
0·025	0·0238	0·0516	4·64
0·050	0		

It is evident from these results that the amylolytic power of diastase, like that of ptyaline, is diminished in proportion as the percentage of alkaline carbonate is increased. Moreover, it would appear by comparison with results previously obtained* that diastase is far more susceptible to the action of sodium carbonate than ptyaline, and also that dilution of the malt extract does not so materially affect the retarding action of the different percentages of alkaline carbonate as in the case of saliva. Both of these results, however, may be due either to the presence of a larger amount of proteid matter in the saliva or to the presence of a larger proportion of ferment, or in fact, to both. It is noticeable in both series of experiments that the amylolytic power of the ferment after gradually diminishing appears to receive a sudden check, which in the larger amount of malt extract is produced by just double the percentage of carbonate requisite with the smaller amount of malt. In this way the effect of dilution is apparent and shows moreover that the exact influence of a given percentage of the alkaline carbonate can be designated only for a definite mixture.

Destructive action of sodium carbonate on diastase.

In order to ascertain how far the retarding action of sodium carbonate is due to destruction of the ferment, the following experiment was tried. Six mixtures were made as follows:

	1	2	3	4	5	6
Malt extract ...	30 c.c.	30 c.c.	30 c.c.	30 c.c.	30 c.c.	30 c.c.
Na_2Co_3 sol.	0 "	1·25 " 0·1%	2·5 " 0·1%	2·5 " 0·5%	5 " 0·5%	10 " 0·5%
H_2O	20 "	18·75 "	17·5 "	17·5 "	15 "	10 "
	50	50	50	50	50	50
Per cent. Na_2Co_3	0	0·0025	0·005	0·025	0·05	0·1

These were warmed at 40° C. for 1 hour, then neutralizing and equalizing mixtures were added as follows:

* Chittenden and Smith.

			1	2	3	4	5	6
0·1 per cent.		HCl	0	0·42 c. c.	0·85 c. c.	4·25 c. c.	8·5 c. c.	16·95 c. c.
0·5	"	Na_2Co_3 *	10 c. c.	9·75	9·5	7·5	5·0	0
0·1	"	HCl *	16·95	16·55	16·1	12·7	8·45	0

The six solutions were now exactly alike; neutral to test papers and contained the same amounts of diastase and sodium chloride. They had, however, been exposed to the action of the above percentages of sodium carbonate for 1 hour at 40° C. Their amylolytic power was now determined in the usual manner (action on 1 gram of starch in a total dilution of 100 c. c.) with the following results:

No.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
1	0·1739 gram.	0·3558 gram.	32·02 per cent.
2	0·1737	0·3554	31·97
3	0·1745	0·3570	32·13
4	0·0341	0·0722	6·49
5	0·0319	0·0678	6·10
6	0·0281	0·0602	5·41

No destructive action is apparent until 0·025 per cent. sodium carbonate is reached; warming the malt extract with 0·005 per cent. sodium carbonate causes no destruction whatever, while with 0·025 per cent. destruction is very great. The amount of malt extract (30 c. c.) experimented with, being the same as was used in determining the influence of alkaline carbonate on the amylolytic power of the ferment, the two series of results are directly comparable and show plainly that the retarding action of small percentages, in the present case up to 0·005 per cent., is due to simple retardation without destruction of the ferment. Beyond this point, however, as in the presence of 0·025 per cent. the greatly diminished amylolytic action is due to destruction of the ferment. Hence it would appear that in the case of the diastase of malt the destructive action of sodium carbonate is out of all proportion to its retarding action. This apparent difference, however, between diastase and the ptyaline of saliva is due, as we shall show later on, to the comparatively small amount of proteid matter in the malt extract. Saliva very greatly diluted, so that the percentage of proteid matter is reduced to a minimum, shows similar results.

Influence of neutral peptone on the amylolytic action of diastase.

It was demonstrated some time since,† that the presence of neutral peptone tends to increase the amylolytic action of neutral saliva.

* The two equalizing mixtures were united before being added to the main solutions.

† Chittenden and Ely, Amer. Chem. Jour., vol. iv, 107.

Langley and Eves* have confirmed this statement, although they do not believe in the theory of a direct stimulation of the ferment, advanced by one of us. We find now that neutral peptone added to a neutral solution of malt diastase, similarly increases its amylolytic action; the increase being even greater than noticed in the case of neutral saliva. Two series of experiments were tried with the following results; the peptone used being made perfectly neutral with a dilute solution of sodium carbonate.

a. with 15 c. c. of the standard malt extract.

Peptone.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1140 gram.	0·2320 gram.	20·88 per cent.
0·1 per cent.	0·1545	0·3154	28·38
0·2	0·1512	0·3084	27·75
0·3	0·1494	0·3048	27·43
0·5	0·1457	0·2970	26·73
1·0	0·1427	0·2910	26·19

b. with 30 c. c. of the standard malt extract.

0	0·1785 gram.	0·3654 gram.	32·88 per cent.
0·1 per cent.	0·1847	0·3772	33·94
0·3	0·1912	0·3916	35·24

Peptone causes increased amylolytic action throughout; with 15 c. c. of malt extract, the smallest amount of peptone gives the greatest acceleration, which slowly diminishes as the percentage of peptone is increased; with 30 c. c. of malt extract, however, acceleration, which is much less than in the preceding series, increases with the increase in peptone. It is hard to find any reason for this acceleration in amylolytic action, other than a direct stimulation of the ferment.

Influence of sodium carbonate on the amylolytic action of diastase in the presence of proteid matter.

Proteid matter tends to prevent the retarding action of sodium carbonate on this ferment, as in the case of the salivary ferment. Thus, the addition of neutral peptone to a malt extract allows vigorous amylolytic action to take place in the presence of percentages of sodium carbonate, which alone would completely destroy the ferment. The following experiments, using 15 c. c. of the standard malt extract in each instance, illustrate the influence of peptone on the action of sodium carbonate.

* Journal of Physiology, vol. iv, No. 1.

With 0·5 per cent. neutral peptone.

Na_2CO_3.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1388 gram.	0·2828 gram.	25·45 per cent.
0·001 per cent.	0·1443	0·2942	26·47
0·002	0·1431	0·2918	26·27
0·003	0·1485	0·3030	27·27
0·004	0·1404	0·2860	25·74
0·005	0·1406	0·2864	25·77
0·010	0·1317	0·2682	24·13

It is thus seen that the presence of 0·5 per cent. of neutral peptone entirely prevents the retarding action of the several percentages of sodium carbonate, except in the last experiment of the series where slight retardation is apparent. It is to be remembered here that even 0·005 per cent. of sodium carbonate alone, almost completely stops the action of the ferment. What at first sight appears to be strange in this last series of experiments, is that the first three percentages of sodium carbonate cause a gradual increase in amylolytic action over that of the neutral fluid plus like percentage of peptone. The explanation of this, however, is quite simple. In studying the influence of neutral peptone on the action of the ferment (15 c. c. malt) it was found that the greatest acceleration of amylolytic action was obtained with the smallest percentage of peptone, and moreover that ferment action diminished in proportion as the peptone was increased. Now peptone undoubtedly prevents the action of sodium carbonate on the ferment by combining with it, forming an alkaline carbonate-proteid compound, possessed of but little retarding action; hence in the above experiment the first action of the smallest percentages of sodium carbonate is to diminish the amount of free peptone, thus causing slight acceleration; further on, however, the increased amount of alkaline-proteid body formed, counteracts the accelerating influence of the free peptone, when gradual retardation commences; finally, increase in the percentage of sodium carbonate leads to the presence of free sodium carbonate, when amylolytic action comes to a sudden standstill. This point being reached, increasing the percentage of peptone prevents the stoppage of ferment action. This is well illustrated by the following series of experiments, using larger percentages of both peptone and sodium carbonate, but 15 c. c. of the malt extract, as before.

Neutral peptone.	Na_2CO_3.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
1·0 per cent.	0	0·2078 gram.	0·4268 gram.	38·41 per cent.
1·0	0·010 per cent.	0·1583	0·3234	29·10
1·0	0·025	0·1529	0·3122	27·09
1·0	0·050	0·1351	0·2754	24·78
1·0	0·100	0·0086	0·0209	1·88
2·0	0·100	0·1341	0·2730	24·57

Here, as before, the retarding action of sodium carbonate is held in check by the peptone, although there is slight retardation due to the alkaline-proteid body formed. Finally, the percentage of sodium carbonate being increased beyond the necessary proportion of peptone, there is a sudden cessation of ferment action. Increasing the amount of peptone, however, prevents this retarding action; evidently the alkaline-proteid body is without much effect on the ferment, only slowly diminishing its amylolytic power.

Influence of acid-proteids on the amylolytic action of diastase.

Falk has noticed that peptone prevents to a certain extent, the retarding action of dilute acid on this ferment; no quantitative results, however, have been recorded, nor has any attempt been made to ascertain whether said action is due to simple retardation, or destruction of the ferment, or both. The action of acids, whether free or combined with proteid matter, on the diastase of malt is particularly important, in view of the rapid passage of the ferment into the stomach when taken in therapeutical preparations. Its ultimate fate must depend in great part upon the action of free and combined (proteid) hydrochloric acid upon it. It is, moreover, important to compare the behavior of the ferment in this respect, with the amylolytic ferment of saliva.

An aqueous extract of malt prepared as described, contains but little proteid matter; as a rule 2·0–2·5 c. c. of 0·1 per cent. hydrochloric acid are required to completely saturate the proteid matter contained in 30 c. c. of the neutral malt extract. This point was ascertained by use of the tropaeolin test for free acid as recommended by Danilewsky.* Thus, by way of illustration, in one instance 30 c. c. of carefully neutralized malt extract required the addition of 3·3 c. c. 0·1 per cent. HCl to give the tropaeolin reaction for free acid, and since nothing smaller than 0·003 per cent. free HCl can be detected by this method, it follows, making the proper deduction, that 2·3 c. c. of 0·1 per cent. HCl are required to completely saturate

* Centralbl. Med. Wiss., 1880; also description in Trans. Conn. Acad., vol. vi, p. 360.

the proteid matter in 30 c. c. of the extract, which would then contain 0·0023 per cent. combined HCl in the form of acid-proteids.*

Our first experiments were made to ascertain the influence of small percentages of combined acid on the action of the ferment, viz: the influence of such additions of dilute acid to the neutral malt extract as would completely saturate the proteid matter present, without giving any *free* acid whatever. The results plainly show that the addition of very small quantities of dilute hydrochloric acid to a neutral solution of diastase increases the amylolytic action of the ferment. Following are a few of the results obtained.

A. Malt extract, neutral to test papers, required 2·4 c. c. 0·1 per cent. HCl to completely saturate the proteid matter in 30 c. c.; the fluid was then acid to test papers but contained no free acid.

a. with 30 c. c. of the malt extract.

Per cent. combined HCl.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1630 gram.	0·3332 gram.	29·98 per cent.
0·0024	0·1749	0·3578	32·19

b. with 15 c. c. of the malt extract.

0	0·1285 gram.	0·2516 gram.	23·54 per cent.
0·0012	0·1460	0·2976	26·78

B. Malt extract, neutral to test papers, required 3·1 c. c. 0·1 per cent. HCl to saturate the proteid matter in 30 c. c. of the extract.

a. with 30 c. c. of the malt extract.

Per cent. combined HCl.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1595 gram.	0·3258 gram.	29·32 per cent.
0·0031	0·1761	0·3602	32·41

b. with 15 c. c. of the malt extract.

0	0·1319 gram.	0·2686 gram.	24·17 per cent.
0·00155	0·1599	0·3266	29·39

C. With 30 c. c. of malt extract; two distinct preparations.

Per cent. combined HCl.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1575 gram.	0·3214 gram.	28·92 per cent.
0·0023	0·1766	0·3612	32·50
0	0·1681	0·3438	30·94
0·0023	0·1770	0·3620	32·58

* Doubtless, however, it is not wholly as HCl-proteid, since the extract frequently contains a trace of sodium lactate.

Such a degree of uniformity in the results, makes it evident that the slight accelerating action of the acid-proteid is a constant one under the above conditions, and indeed all of our results in this direction clearly indicate such to be the case.

Although acid-proteid thus accelerates amylolytic action, it is still able under slightly different conditions to retard the action of the ferment and even cause destruction; thus by warming the amount of ferment (30 c. c. malt extract), used in the preceding experiment, at 40° C. for 1 hour with the acid necessary to saturate the proteids, in a total volume of 50 c. c., thus doubling the *percentage* of acid, amylolytic action was found on subsequent neutralization and testing with starch paste, to be very much diminished. The following results obtained with the two malt extracts used in C illustrate this point:

	a.	*b.*	*c.*	*d.*
Per cent. HCl	0	0·0046	0	0·0046
Malt extract	30 c. c.	30 c. c.	30 c. c.	30 c. c.
0·1 per cent. HCl	0	2·3 "	0	2·3 "
H_2O	20 "	17·7 "	20 "	17·7 "
	50·0 c. c.	50·0 c. c.	50·0 c. c.	50·0 c. c.

These solutions were warmed at 40° C. for 1 hour, then neutralizing and equalizing mixtures were added, after which amylolytic action was determined by adding starch paste, diluting to 100 c. c., warming at 40° C. for 30 minutes, etc., with the following results, expressed in the percentage of starch converted into sugar.

a.	*b.*	*c.*	*d.*
30·24	21·74	29·25	22·96

Hence, it is apparent that under these conditions, acid-proteids may exert a destructive action on the ferment. This destructive action takes place with considerable degree of rapidity, as is shown by the following experiment, which at the same time illustrates the accelerating action of smaller percentages of combined acid and confirms the preceding experiments.

The neutral malt extract required 2·4 c. c. 0·1 per cent. HCl to combine with the proteid matter in 30 c. c.

	Destructive action.			Accelerating action.	
	1.	*2.*	*3.*	*4.*	*5.*
Malt extract	30 c. c.	30 c. c.	30 c. c.	30 c. c.	30 c. c.
0·1 per cent. HCl	0	2·4 "	2·4 "	0	2·4 "
H_2O	20 "	17·6 "	17·6 "	70* "	70* "
	50 "	50 "	50 "	100	100
Per cent. HCl	0	0·0048	0·0048	0	0·0024

* Containing one gram of starch.

The amylolytic action of 4 and 5 was tested directly by warming the mixtures at 40° C. for 30 minutes and then determining the amount of reducing bodies. No. 1 (the control) was warmed at 40° C. for 30 minutes, while No. 2 was warmed at the same temperature for 15 minutes and No. 3 for 30 minutes. Neutralizing and equalizing mixtures were added as follows:

	1.	*2.*	*3.*
0·1 per cent. Na_2CO_3...	0	3·5 c. c.	3·5 c. c.
0·1 per cent. HCl.....	2·4 c. c.	0	0
0·1 per cent. Na_2CO_3...	3·5 "	0	0

All three were then mixed with one gram of starch and made up to 100 c. c. in order to determine amylolytic action; each solution was neutral and contained the same quantity of sodium chloride as well as the same amount of ferment and starch. Following are the results of all five:

No.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
1	0·1630 gram.	0·3332 gram.	29·98 per cent.
2	0·1253	0·2554	22·98
3	0·1195	0·2434	21·90
4	0·1628	0·3322	29·89
5	0·1749	0·3578	32·19

It is thus seen that with this amount of ferment, 0·0024 per cent. combined acid causes an acceleration in amylolytic action (Nos. 4 and 5) amounting to over 2 per cent. in the quantity of starch converted, while warming the same amount of ferment with the same amount of acid, but under a less degree of dilution, causes a destruction of the ferment amounting to 7 per cent. in the conversion of the starch; 15 minutes longer at 40° C. causes only a slightly increased destruction.

Influence of acid-peptone.

By increasing the amount of proteid matter, larger percentages of hydrochloric acid can be added to a malt extract without retarding the action of the ferment or even interfering with the accelerating action of the smaller percentages. As previously stated, Falk has shown that peptone prevents to a certain extent the retarding action of hydrochloric acid, but it does more than this, it causes acceleration in ferment action not only in neutral solution, as already shown, but in an acid solution likewise, provided there is an excess of peptone present, in which case the acid-peptone compound formed, causes greater acceleration than the same percentage of peptone alone would do if added to a neutral solution of the ferment. The follow-

ing experiments, which we have repeated many times, testify to the accuracy of this statement:

Per cent. Peptone.	Per cent. combined HCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0·2	0	0·2118 gram.	0·4356 gram.	39·20 per cent.
0·2	0·0003	0·2168	0·4460	40·14
0·2	0·0005	0·2115	0·4348	39·13
0·2	0·0030	0·2165	0·4454	40·18
0·2	0·0050	0·2175	0·4478	40·30
0·2	0	0·1730	0·3540	31·86
0·2	0·0003	0·1726	0·3532	31·78
0·2	0·0005	0·1802	0·3688	33·17
0·2	0·0010	0·1794	0·3672	33·04
0·2	0·0030	0·1765	0·3610	32·49
0·2	0·0050	0·1775	0·3632	32·68

Acceleration in amylolytic action is here quite noticeable; the peptone, however, is in considerable excess. As the peptone approaches saturation, retardation commences as is shown by the following experiments; such a point being reached, however, retardation can be completely prevented by increasing the amount of peptone.

Per cent. Peptone.	Per cent. combined HCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0·2	0	0·1716 gram.	0·3508 gram.	31·57 per cent.
0·2	0·008	0·1732	0·3424	30·81
0·2	0·010	0·1631	0·3334	30·00
0·2	0·012	0·1442	0·2940	26·46
0·5	0	0·1633	0·3338	30·04
0·5	0·012	0·1652	0·3376	30·38
0·5	0·025	0·1170	0·2384	21·45

The peptone employed in this last experiment required 11·8 c. c. of 0·1 per cent. hydrochloric acid to completely saturate 0·2 gram, consequently in the fourth experiment the peptone was more than completely saturated with acid, but the amount of free acid could have been only 0·0002 per cent. In the last experiment, however, retarding action is due wholly to acid-peptone, no free acid at all being present. Increasing the percentage of combined acid still further, the proteid matter at the same time being just saturated, causes far greater retardation in the action of the ferment.

Per cent. Peptone.	Per cent. combined HCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0·5	0	0·1607 gram.	0·3282 gram.	29·53 per cent.
0·5	0·012	0·1661	0·3394	30·54
0·5	0·015	0·1670	0·3412	28·70
0·5	0·025	0·1202	0·2448	22·03
0·5	0·035*	0·0317	0 0674	6·06

* With this percentage the peptone is exactly saturated with acid.

All of these results show acceleration under the influence of small percentages of combined acid, followed, as the percentages are increased, by decided retardation, thus agreeing with the results previously obtained with the salivary ferment.

The destructive action of small percentages of acid-peptone on the ferment is not great in the presence of an excess of peptone, but as the peptone approaches saturation its destructive power is increased, and when completely saturated with acid, a moderate amount of peptone so combined will quickly and completely destroy the ferment. This is plainly shown in the following series of experiments:

	1.	*2.*	*3.*	*4.*
Malt extract	15 c. c.	15 c. c.	15 c. c.	15 c. c.
Peptone sol.*	20	20	20	20
HCl 0·1 per cent.	0	1	2	4
H_2O	15	14	13	11
	50	50	50	50
Peptone	0·1 per cent.	0·1 per cent.	0·1 per cent.	0·1 per cent.
Combined HCl	0	0·002	0·004	0·008

These mixtures were warmed at 40° C. for 30 minutes. 20 c. c. of the peptone solution together with 15 c. c. of the malt extract required 4·0 c. c. of 0·1 per cent. hydrochloric acid to saturate the proteid matter, consequently in the above mixtures, 2 and 3 contained a large excess of uncombined peptone, while in 4 the proteid matter was just saturated.

Neutralizing and equalizing mixtures were added to each as follows:

		1.	*2.*	*3.*	*4.*
0·1 per cent.	Na_2CO_3	0	1·5 c. c.	3 c. c.	5·9 c.c.
0·1 "	Na_2CO_3	5·9 c. c.	4·4	2·9	0
0·1 "	HCl	4·0	3·0	3·0	0

The solutions were now, on being diluted to 100 c. c., in every respect equal. Their amylolytic power on being tested with starch paste was as follows:

No.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
1	0·1595 gram.	0·3258 gram.	29·32 per cent.
2	0·1527	0·3118	28·06
3	0·1239	0·2522	22·69
4	trace.		

Hence, when the proteid matter present is only one-quarter saturated with acid, the acid-peptone so formed may exert some destruc-

* The peptone solution contained 0·250 gram peptone in 100 c. c. water and was made neutral to test papers; 20 c. c. therefore contained 0·050 gram peptone.

tive action on the ferment; when half saturated, destructive action is more pronounced; when wholly saturated it is, under the above conditions, complete.

Frequently, such small percentages of combined acid as the above will have no retarding effect whatever on amylolytic action, though if the ferment be warmed for half an hour with the same percentage of peptone and combined acid, its subsequent amylolytic power will be much reduced, owing to a partial destruction of the ferment. This is well illustrated by the following series of experiments:

	A.			B.		
	1.	*2.*	*3.*	*4.*	*5.*	*6.*
Malt extract.....	15 c. c.	15 c. c.	15 c. c.	15 c. c.	15 c. c.	15 c. c.
0·1 per cent. HCl..	0	1	2	0	2	4
Peptone sol. 0·5%..	10	10	10	20	20	20
H_2O.....	25	24	23	65*	63*	61*
	50	50	50	100	100	100
Combined HCl....	0	0·002%	0·004%	0	0·002%	0·004%
Peptone	0·1%	0·1	0·1	0·1%	0·1	0·1

Nos. 4, 5 and 6 were warmed directly, with the starch, for 30 minutes at 40° C. and the reducing bodies determined. Nos. 1, 2 and 3 were warmed at 40° C. also for 30 minutes, then neutralizing and equalizing mixtures were added, the solutions diluted to 100 c. c. and warmed with 1 gram of starch for 30 minutes at 40° C. The following results show the amylolytic power of the six solutions:

	No.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
A.	1	0·1482 gram.	0·3024 gram.	27.21 per cent.
	2	0·1412	0·2876	25·88
	3	0·1351	0·2754	24·78
B.	4	0·1594	0·3256	29·30
	5	0·1628	0·3324	29·91
	6	0·1605	0·3282	29·52

In series A, we see a gradual decrease in the amylolytic power of the solutions; this can be due to nothing but the destructive action of the acid-peptone compound, for the solutions are in every respect alike, being in the same degree of dilution and containing the same amount of sodium chloride, etc. The destruction is not great, as the peptones are in considerable excess. In series B, where the ferment is exposed to the direct action, in the presence of the starch, of the same percentage of peptone and acid there is no retardation of

* Containing 1 gram of starch.

amylolytic action whatever; presumably because of the rapid action of the ferment and the slow retarding action of the acid-peptone.

Influence of free acid on the amylolytic action of diastase.

As might be expected, free hydrochloric acid, even in very small quantity at once stops the amylolytic action of this ferment, quickly destroying it. It is interesting, however, to compare the action of very small percentages of free acid with results obtained in like manner with the salivary ferment. The first experiment gave the following results: the malt extract used, required per 30 c. c., 2·4 c. c. 0·1 per cent. hydrochloric acid to saturate the proteid matter.

Per cent. combined HCl.	Per cent. free HCl.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0	0·1378 gram.	0·2808 gram.	25·27 per cent.
0·0024	0	0·1584	0·3236	29·12
0·0024	0·001	0·1486	0·3032	27·29
0·0024	0·003	0·0805	0·1642	14·77

With another malt extract not so active, but containing the same percentage of acid-proteids, the following results were obtained:

Free HCl.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·0780 gram.	0·1592 gram.	14·32 per cent.
0·0003 per cent.	0·0719	0·1470	13·23
0·0005	0·0631	0·1294	11·64
0·0020	0·0380	0·0796	7·16
0·0030	0·0091	0·0222	1·99

From these two series of results it is quite evident that a very small percentage of free hydrochloric acid will stop the amylolytic action of this ferment. The main action of the acid is that of destruction, killing the ferment very quickly. The following is a sample of several experiments tried, to ascertain how far retardation is due to destruction of the ferment.

	1.	*2.*	*3.*	*4.*
Neutral malt extract	30 c. c.	30 c. c.	30 c. c.	30 c. c.
0·1 per cent. HCl to saturate proteids	2·2 "	2·2 "	2·2 "	2·2 "
0·1 per cent. HCl for free acid	0	0·5 "	1·0 "	1·5 "
H_2O	17·8 "	17·3 "	16·8 "	16·3 "
	50 "	50 "	50·0 "	50·0 "
Per cent. free HCl	0	0·001	0·002	0·003

These solutions were warmed at 40° C. for 30 minutes, then neutralizing and equalizing mixtures were added and the amylolytic power determined. No. 1 converted the usual amount of starch into sugar, but No. 2 showed only a trace of amylolytic power and Nos. 3 and 4 none at all. Evidently then 0·001 per cent. of free acid had

all but completely destroyed the ferment by 30 minutes warming at 40° C.

In conclusion then, we have to notice a greater susceptibility on the part of this ferment to the action of acid-proteids and free acid than the salivary ferment. Whether this latter point constitutes any real difference, it is hard to say, since the apparent increase in amylolytic action noted in the presence of traces of free acid in the case of saliva (0·0001–0·0006 per cent. free HCl) involve such small quantities as to make the results somewhat questionable,* since such very small additions of acid might perhaps be used up by the phosphates or other salts present. But taking the evidence of the results and comparing them with results obtained in like manner with the diastase of malt, it would certainly appear that the latter is more susceptible to the action of free acid than the salivary ferment, though both are very readily destroyed by a few thousandths of one per cent. of free HCl.

In other respects, the ferment of malt behaves similarly to the ferment of saliva; both act better in a neutral than in an alkaline solution; proteid matter too, prevents the retarding action of alkaline carbonate and thus, as in the case of saliva, the action of a given percentage of sodium carbonate on diastase is dependent in part, upon the concentration of the fluid and the consequent amount of proteid matter present. Neutral peptone, moreover, exerts a direct stimulating effect on the amylolytic action of neutral diastase. Greatest amylolytic action, as in the case of saliva, is, however, observed in the presence of proteid matter partially saturated with acid, but larger percentages of acid-proteids may cause complete destruction of the ferment. The accelerating action of proteid matter is in great part due to its power of combining with both acid and alkaline carbonate, but in addition we cannot but recognize a direct stimulation of the ferment, as in the action of neutral peptone on a neutral solution of diastase.

Lastly, it is evident from these results, that diastase taken into the stomach must sooner or later be completely destroyed, by either the free acid or the large percentage of acid-proteids; but in the first stage of digestion, in the absence of free acid and under the protecting influence of proteid matter the conversion of starch into sugar may still go on, though soon destined to feel the effects of the gradually increasing percentage of combined acid.

* See Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 370.

Influence of certain Therapeutic and Toxic Agents on the amylolytic action of Saliva. By R. H. Chittenden and H. M. Painter, B.A., Ph.B.

Few attempts have been made to ascertain, experimentally, the influence of therapeutic and toxic substances on amylolytic action. Yet in view of the important part which the ferment of saliva plays in the digestive processes of the body and in view likewise of the great susceptibility of the ferment, it would seem especially desirable to obtain accurate data regarding the effects of many substances on its amylolytic power.

While many laborious investigations have, from time to time, been undertaken to ascertain the influence of some one or more substances on the metabolism of the body, the influence of the same substances on the digestive processes has apparently been very little considered, with the exception, however, of the more common alkali and alkali-earth salts. Likewise too, the possible action of many toxic substances on the digestive processes, as in chronic cases of poisoning, has with a few exceptions been almost entirely ignored; yet in both of these instances it is possible that much light might be obtained by a knowledge of the influence of individual substances upon proteolytic and amylolytic action.*

With these thoughts in mind, the present investigation was undertaken, and the results which we present here plainly show the importance of the work.

In selecting substances for study, we have chosen not only those noted for therapeutic or toxic power, but also those possessed of antiseptic or germicidal properties; our object being to see how far the unformed ferment of the saliva corresponds, in its behavior towards these bodies, with the formed or organized ferments.† More-

* An interesting table of comparisons by Wernitz shows the relative action of several therapeutic agents, on the various enzymes of physiological interest.—Brunton's Pharmacology, p. 86.

† A difference in action by the same substance upon formed and unformed ferments is, as stated by Brunton, a fact of great importance, for upon it may depend a useful application of the substance in medicine; thus creosote, which has but a slight action upon pepsine and ptyaline, will kill bacteria in a dilution of 1 to 1000, and thus this agent can be used to arrest fermentation in the stomach depending on the presence of low organisms, while the proteolytic action of the digestive ferment is but little interfered with.—Brunton, p. 87.

over, only neutral bodies could be experimented with, since the smallest quantity of either free acid or alkali would exert its own peculiar destructive action on the ferment.* In view of this fact also, we have invariably used chemically pure salts and those frequently recrystallized to be sure of the absence of deleterious impurities.

Method employed.

A few preliminary experiments clearly indicated that the presence of very small percentages of foreign substances exercise a decided effect on the amylolytic action of saliva, and thus the investigation resolved itself into a study, not of the percentages requisite to *completely* hinder the power of the ferment, under given conditions, but of the relative action of small percentages on the amylolytic power of the ferment. This seemed to us the more important, since we soon found that substances which, present in comparatively large amount tended to hinder amylolytic action, would when present in small quantities actually increase the activity of the ferment. Hence, we deemed it best to use accurate quantitative methods for determination of amylolytic action; such as would indicate small variations with certainty.

The experiments were made in series, in which one digestion of each series served as a control for comparison. The volume of each digestive mixture was 100 c. c., in which was present 1 gram of perfectly neutral potato starch, previously boiled with a portion of the water, 10 c. c. of a diluted neutral saliva† and a given quantity of the substance to be experimented with. The mixtures were warmed at 40° C. for 30 minutes, after which further action of the ferment was stopped by heating the solution to boiling. The extent of amylolytic action was then ascertained by determining in one-fourth of the solution, the amount of reducing substances by Allihn's‡ gravimetric method. From the amount of reduced copper thus obtained, the total amount of reducing bodies was calculated (as dextrose), from which in turn was calculated the percentage of starch converted.

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* Chittenden and Smith, Transactions Conn. Acad. Arts and Sciences, vol. vi, p 343.

† The saliva was human, mixed saliva, freshly collected. It was prepared for use by being filtered, made exactly neutral, then diluted in the proportion of 1 : 5. Thus in each digestion there were present 2 c. c. of undiluted saliva.

‡ Zeitschrift für Analytische Chemie, Jahrgang xxii, p. 448.

Mercuric chloride.

Sternberg* places mercuric chloride first in the list of germicides; its presence to the extent of 0·003 per cent. being sufficient to prevent the development of the micrococcus of pus, while 0·005 per cent. destroys the vitality of the same bacterial organism.

To our surprise the salt acts even more energetically on the unorganized ferment of the saliva, as the following results show:

$HgCl_2$	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·2385 gram.	0·4920 gram.	44·28 per cent.
0·0005 per cent.	0·1277	0·2500	22·50
0·0010	0·0925	0·1880	16·92
0·0020	0·0395	0·0824	7·41
0·0030	0·0060		
0·0040	0		

It is evident that the ferment of saliva is very susceptible to the action of this poison, and we have repeated the experiment, using still smaller percentages, with the following results:

$HgCl_2$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1635 gram.	0·3340 gram.	30·06 per cent.
0·0001 per cent.	0·1610	0·3288	29·59
0·0002	0·1570	0·3204	28·83
0·0003	0·1545	0·3152	28·36

The smallest possible addition, therefore, of mercuric chloride diminishes the amylolytic power of saliva, in proportion to the amount of mercury salt added.

Mercuric bromide, mercuric iodide and mercuric cyanide.

These salts of mercury, vigorous in their action as poisons, and the two former as germicides likewise, would be expected from analogy to act similarly to the chloride. Such we find to be the case with the bromide and iodide, but with the cyanide there is to be noticed, to a slight extent, an action which we find common to many substances, viz: increasing the amylolytic power of the ferment

* Amer. Jour. Med. Sciences, April, 1883, p. 321.

For the action of the various salts studied in this work, on the organized ferments, see also Marcus and Pinet in Compt. Rend. Soc. de Biolog., 1882, pp. 718–724, or abstract in Jahresbericht für Thierchemie, 1882, p. 515; also Ch. Richet in Compt. Rend., vol. xcvii, pp. 1004–1006, or in Jahresbericht für Thierchemie, 1883, p. 418, and Robert Koch, Jahresbericht für Thierchemie, 1881, p. 471, N. Jalan de la Croix, Jahresbericht für Thierchemie, 1881, p. 476. Brunton's Pharmacology, p. 96.

when present in one percentage and diminishing it when the percentage is increased. On account of the insolubility of mercuric iodide and bromide, these salts were dissolved in water containing potassium iodide and sodium chloride respectively, in such proportion that the various digestive mixtures contained the same percentages of these salts as they did of the mercury salts.* Following are the results obtained:

Mercury salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1295 gram.	0·2636 gram.	28·72 per cent.
$HgBr_2$			
0·0005 per cent.	0·1150	0·2344	21·09
0·0010	0·0770	0·1572	14·14
0·0020	0·0340	0·0720	6·48
HgI_2			
0·0010	0·1257	0·2560	23·04
0·0020	0·1180	0·2404	21·63
$Hg(CN)_2$			
0·0005	0·1375	0·2800	25·20
0·0010	0·1445	0·2944	26·49
0·0020	0·1242	0·2528	22·75
0·0030	0·1252	0·2552	22·96
0	0·1319	0·2684	24·15
$Hg(CN)_2$			
0·0500	0·1025	0·2084	18·75

In this series of results, it is to be noticed that mercuric bromide is the most energetic in its hindering action; 0·0005 per cent. being even more effective than 0·002 per cent. of the iodide. With the cyanide, however, the first two percentages stimulate or in some way give rise to an increased amylolytic action and even 0·050 per cent. of the salt does not retard the action of the ferment as much as 0·001 per cent. of mercuric bromide.

Cupric sulphate.

With this salt the following results were obtained:

$CuSO_4+5H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1712 gram.	0·3500 gram.	31·50 per cent.
0·0005 per cent.	0·1445	0·2944	26·49
0·0020	0·0530	0·1096	9·86
0·0100	0·0250	0·0540	4·86
0·0250	0		

* Apparently, the double salts so formed act as vigorously as the mercury salt alone could do.

The hindering action of the copper salt is nearly as pronounced as that of mercuric chloride and even more so than the bromide and iodide of mercury.

Lead acetate.

With this salt, the smaller percentages experimented with show a slight stimulating action; but the larger percentages fail to retard the amylolytic action of the ferment as the preceding salts.

$Pb(C_2H_3O_2)_2+3H_2O$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1630 gram.	0·3332 gram.	29·98 per cent.
0·0003 per cent.	0·1642	0·3356	30·20
0·0005	0·1635	0·3340	30·06
0·0010	0·1635	0·3340	30·06
0·0020	0·1595	0·3256	29·30
0·0050	0·1395	0·2840	25·56
0·0100	0·1402	0·2856	25·70

A second series of experiments, with still larger percentages of the lead salt, gave the following results:

$Pb(C_2H_3O_2)_2+3H_2O$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1742 gram.	0·3564 gram.	32·07 per cent.
0·05 per cent.	0·1735	0·3548	31·93
0·10	0·1720	0·3516	31·64
0·30	0·1657	0·3384	30·45
0·50	0·1555	0·3172	28·54
1·00	0·1375	0·2800	25·20
3·00	0·0785	0·1600	14·40
5·00	0·0490	0·1016	9·14

Thus, the presence of even five per cent. of lead acetate fails to completely prevent amylolytic action.

Arsenious oxide.

Owing to the comparative insolubility of this substance in neutral fluids, small percentages only could be experimented with. With these, the following results were obtained:

As_2O_3.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 per cent.
0·0003 per cent.	0·1507	0·3072	27·64
0·0005	0·1537	0·3136	28·22
0·0010	0·1475	0·3004	27·03
0·0020	0·1570	0·3204	28·83
0·0050	0·1390	0·2832	25·48
0·0900	0·1605	0·3276	29·48

Although the results obtained do not wholly accord with each other they still plainly show that arsenious acid, to the extent pres-

ent in these experiments, stimulates the amylolytic action of the ferment; a fact which might be expected, assuming that the acid combines with the proteids of the saliva, for as has been elsewhere* shown, acid-proteids when present in not too large an amount increase the amylolytic action of the salivary ferment.

Schäfer and Böhm† state that arsenious acid has no influence whatever on the conversion of starch into sugar by a glycerine extract of the pancreas. Possibly they sought only for retarding action, or it may be that the pancreatic ferment differs in this respect from the ferment of saliva.

Arsenic acid.

This substance being still more acid than the preceding, might naturally be expected to diminish amylolytic action, when present in quantities which in the preceding would increase the activity of the ferment; and indeed there is to be seen in the results, a slight increase, followed by a rapid decrease of amylolytic action.

H_3AsO_4.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1755 gram.	0·3588 gram.	32·29 per cent.
0·0005 per cent.	0·1765	0·3608	32·47
0·0010	0·1635	0·3340	30·06
0·0030	0·0310	0·0660	5·94
0·0050	0		

With 0·005 per cent. of arsenic acid present in the fluid, no reducing bodies were formed in the thirty minutes of the experiment, but the solution did become clear, showing the formation of soluble products. The same fact was observed in the presence of larger percentages of the acid; the starch solution becoming clear, after the addition of saliva, even in the presence of one per cent. of the acid, although, as before, no reducing bodies were formed.

Ammonium ursenate.

With this salt the following results were obtained:

$(NH_4)_3AsO_4$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1527 gram.	0·3112 gram.	28·08 per cent.
0·0005 per cent	0·1620	0·3308	29·77
0·0010	0·1630	0·3340	30·06
0·0050	0·1675	0·3420	30·78
0·0150	0·1745	0·3568	32·11
0·0250	0·1700	0·3476	31·28

* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

† Abstract in Jahresbericht für Thierchemie, 1872, p. 365.

In a second series, larger percentages were used with the following results:

$(NH_4)_3AsO_4$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1712 gram.	0·3500 gram.	31·50 per cent.
0·05 per cent.	0·1475	0·3004	27·03
0·10	0·1147	0·2332	20·98
0·50	0·0165	0·0368	3·31
1·00	The solution became clear but no reducing bodies were formed.		

With this salt, a very decided stimulation of the ferment is to be observed in the presence of small percentages, while increased amounts of the salt ultimately stop diastatic action.

Potassium antimony tartrate.

Two series of experiments were tried with this salt, with the following results:

$K(SbO)C_4H_4O_6$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1540 gram.	0·3144 gram.	28·29 per cent.
0·001 per cent.	0·1610	0·3288	29·59
0·005	0·1660	0·3392	30·52
0·010	0·1760	0·3600	32·40
0·050	0·1760	0·3600	32·40
0·100	0·1745	0·3568	31·11
0·200	0·1750	0·3580	32·22
0	0·1545	0·3152	28·36
0·10	0·1850	0·3788	34·09
0·30	0·1640	0·3352	30·16
0·50	0·2565	0·5304	47·73
1·00	0·1570	0·3204	28·83
2·00	0·1232	0·2504	22·53
5·00	0·0470	0·0976	8·78

Here we have an illustration, more forcible than with any other salt, of the power possessed by many substances of both increasing and diminishing the action of the ferment. One* of us has for some time held that the addition of very small quantities of hydrochloric acid to neutral saliva tends to increase the amylolytic power of the ferment; that this takes place even when the proteids present are completely saturated with the acid, or in other words when there is present a *very small* amount of *free* acid, provided the acid-proteids are not present in too large an amount. It is well known that free hydrochloric acid, when present to the extent of a few thousandths of one per cent. completely stops the action of the ferment. Langley

* Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 360.

and Eves* make this divergence of action of one and the same substance a ground for questioning the accuracy of such a view, for, say they, "since 0·0015 per cent. HCl decreases amylolytic action it seems very unlikely that 0·0005 per cent. should increase it." The action of many neutral salts here experimented with, where both stimulation and retardation are obtained, plainly show that such a double action, dependent simply on quantity is not an impossible one.

Stannous chloride.

With this salt very marked results were obtained as follows:

$SnCl_2$	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 per cent.
0·0003 per cent.	0·1582	0·3232	29·08
0·0010	The solution became clear, but no reduction.		
0·0050	The starch was not at all altered in appearance.		

Here there is stimulation, followed by rapid and complete stopping of amylolytic action.

Zinc sulphate.

$ZnSO_4+7H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1495 gram.	0·3048 gram.	27·43 per cent.
0·0003 per cent.	0·1490	0·3040	27·36
0·0005	0·1510	0·3088	27·79
0·0010	0·1475	0·3004	27·03
0·0020	0·1440	0·2936	26·42
0·0050	0·1360	0·2772	24·94
0·0100	0·1260	0·2576	23·18
0	0·1375	0·2800	25·20
0·05 per cent.	0·0775	0·1480	13·32
0·10	0·0650	0·1332	11·98
0·30	0·0450	0·0936	8·42
0·44	0		

These two series of experiments plainly show a gradually diminished amylolytic action, as the percentage of the zinc salt is increased, until with 0·4 per cent. a complete stoppage is effected.

Kjeldahl† found a like retarding action on the addition of zinc sulphate to a malt extract.

In this connection it is interesting to note that Sternberg‡ finds zinc sulphate devoid of germicide value, even when used in the proportion of 20 per cent.

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* Journal of Physiology, vol. iv, No. I.

† Jahresbericht für Thierchemie, 1879, p. 382.

‡ Amer. Jour. Med. Sciences, April, 1883, p. 330.

Ferric chloride.

With this salt we obtained the following results:

Fe_2Cl_6.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1740 gram.	0·3560 gram.	32·04 per cent.
0·0005 per cent.	0·1597	0·3260	29·34
0·0020	0·0437	0·0908	8·17
0·0100	0·0095	0·0236	2·12
0·0250	0		

Sternberg states that tincture of ferric chloride is effective as a germicide (upon micrococcus) when present to the extent of 4 per cent. On the unformed ferment of the saliva, it is, as the results show, much more active, its hindering action being directly proportional to the percentage of iron salt present.

Ferrous sulphate.

With this salt of iron quite different results were obtained; and as we wished simply to compare its action with that of the ferric salt, only very small percentages were experimented with.

$FeSO_4+7H_2O$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1245 gram.	0·2532 gram.	22·78 per cent.
0·0005 per cent.	0·1037	0·2108	18·97
0·0020	0·1323	0·2692	24·22
0·0100	0·1365	0·2780	25·02

Here there is decided stimulation with the two larger percentages, while the smallest per cent. shows an apparent decrease of amylolytic action.

Kjeldahl* found that this salt exercised a strong hindering action on the amylolytic ferment of malt.

Potassium permanganate.

Sternberg places this salt next to mercuric chloride in germicide value, it being efficacious in 0·12 per cent. With the unformed ferment of the saliva it is likewise active, although no more so than many other salts experimented with. Following are the results:

$K_2Mn_2O_8$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 per cent.
0·005 per cent.	0·1012	0·2060	18·54
0·025	0		

* Jahresbericht für Thierchemie, 1879, p. 382.

Magnesium sulphate.

With this salt we obtained the following results:

$MgSO_4+7H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1475 gram.	0·3004 gram.	27·03 per cent.
0·025 per cent.	0·1597	0·3260	29·34
0·500	0·0510	0·1056	9·50

Here, there is a slight increase of diastatic action with the smallest percentage, while 0·5 per cent. of the salt greatly retards the action of the ferment.

Pfeiffer* has likewise noticed the retarding effect of this salt on salivary digestion.

Potassium cyanide.

This salt, so powerful as a poison, was found to have a decided effect also on the salivary ferment, causing a rapid decrease in amylolytic action.

KCN.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1245 gram.	0·2532 gram.	22·78 per cent.
0·0005 per cent.	0·1080	0·2200	19·80
0·0010	0·0896	0·1828	16·45
0·0030	0·0330	0·0700	6·30

With 1·0 per cent. and even with 5·0 per cent. of potassium cyanide, the starch solutions became clear on the addition of saliva, showing that the ferment was able to effect some change, although in neither case were any reducing bodies formed.

It is our intention at some future time, to study the exact nature of the products formed under such conditions. The ferment appears to be peculiarly affected; for while a very small percentage of a substance like potassium cyanide or borax will completely prevent the formation of reducing bodies, increasing the amount of substance added a hundred-fold, has no effect on the clearing up of the starch solution by the ferment. Some light may be thrown upon the nature of the ferment or its mode of action.

Potassium ferrocyanide.

A preliminary experiment showed that this salt was less active than the cyanide and therefore larger percentages were used, with the following results:

$K_4Fe(CN)_6+3H_2O$.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1417 gram.	0·2884 gram.	25·96 per cent.
0·025 per cent.	0·1497	0·3052	27·46
0·100	0·1375	0·2800	25·20
0·250	0·1025	0·2084	18·80

* Centralbl. Med. Wiss., 1885, p. 328, abstract.

Here, unlike the cyanide, there is stimulation of the ferment. Retardation of amylolytic action requires much larger percentages; thus 1·0 per cent. of ferrocyanide completely prevented the formation of reducing bodies, although soluble starch was apparently formed, as also in the presence of 5·0 per cent. of the salt.

Potassium ferricyanide.

The action of this salt is almost identical with that of the ferrocyanide.

$K_6Fe_2(CN)_{12}$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1417 gram.	0·2884 gram.	25·96 per cent.
0·025 per cent.	0·1515	0·3088	27·79
0·100	0·1295	0·2636	23·72
0·250	0·0975	0·1984	17·85

Like the ferrocyanide, this salt in 1·0 and 5·0 per cent. solutions allows the partial conversion of starch into soluble products, but no reducing bodies are formed.

Potassium nitrate and potassium chlorate.

Potassium salt.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1513 gram.	0·3080 gram.	27·72 per cent.
KNO_3.			
0·20 per cent.	0·1550	0·3164	28·47
0·50	0·1528	0·3108	27·97
1·00	0·1462	0·2976	26·78
$KClO_3$.			
0·20	0·1581	0·3228	29·05
0·50	0·1580	0·3228	29·05
1·00	0·1600	0·3268	29·41

With 5·0 per cent. of the salts, the following results were obtained:

Potassium salt.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1672 gram.	0·3416 gram.	30·74 per cent.
KNO_3 (5·0 pr. ct.)	0·1559	0·3176	28·58
$KClO_3$ (5·0 pr. ct.)	0·1351	0·2752	24·76

With these two salts it is very obvious that small fractions of one per cent. decidedly increase amylolytic action and that potassium chlorate is the more energetic of the two in this respect. With one per cent. of the salts, potassium chlorate still shows increased action, while the nitrate causes a decrease of amylolytic activity; in the presence of 5 per cent. of the salts, on the other hand, potassium chlorate causes the greatest decrease in ferment action.

Of these two oxidizing agents, potassium chlorate does not appear to have been hitherto experimented with, but with potassium nitrate

O. Nasse* found increased amylolytic action with human saliva in the presence of 4·0 per cent. of the salt. Possibly this difference in our results is dependent in part upon difference in the relative amount of salt and ferment.

Sodium tetraborate [$Na_2B_4O_7+10H_2O$].

With this salt experiments were tried with quantities varying from 0·050 to 3·0 per cent. and in each instance the starch was dissolved, but no reducing bodies whatever were formed. Dumas† has previously noted a like retarding effect on the diastatic action of emulsin, diastase and other like ferments. Sternberg states that this salt is without germicide value, even though used in a saturated solution; its antiseptic power, i. e. its capacity for preventing the multiplication of bacterial organisms, is, however, considerable.

Potassium bromide and potassium iodide.

These two common therapeutic agents gave the following results:

Salt used.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1483 gram.	0·3020 gram.	27·18 per cent.
KBr.			
0·5 per cent.	0·1566	0·3192	28·72
3·0	0·1450	0·2956	26·60
5·0	0·1314	0·2668	23·51
KI.			
0·5	0·1550	0·3164	28·47
3·0	0·1557	0·3172	28·54
5·0	0·1467	0·2984	26·85

Both of these salts show a stimulating action which is more persistent in the case of the iodide than with the bromide; 5·0 per cent. of the bromide causes a marked diminution of amylolytic action.

Sodium chloride.

Previous experiments have been tried with this salt by several investigators, notably by O. Nasse‡ and E. Pfeiffer.§ The former found that the presence of 4·0 per cent. of the salt [the only percentage experimented with] caused an increase in the ferment action of saliva [128 : 100]; the latter experimenter likewise found that the

* Pflüger's Archiv. für Physiologie, vol. xi, p. 150.

† Berichte der deutsch. Chem. Gesell., vol. v, p. 826.

‡ Pflüger's Archiv für Physiologie, vol. xi, p. 155.

§ Centralbl. med. Wiss., 1885, p. 329.

presence of the salt, in concentrations up to 2 per cent., greatly increased the amylolytic action of saliva.

Our results with different percentages are as follows:

NaCl.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1660 gram.	0·3392 gram.	30·52 per cent.
0·3 per cent.	0·1765	0·3608	32·47
0·5	0·1750	0·3580	32·22
1·0	0·1715	0·3504	31·53
2·0	0·1715	0·3504	31·53
3·0	0·1770	0·3620	32·58
5·0	0·1630	0·3332	29·98

These accord with the results mentioned above and show, moreover, that with 5·0 per cent. of the salt, hindering action just commences. Increasing the amount of salt beyond this point, however, only slowly diminishes the action of the ferment; thus, in the presence of 10·0 per cent. of the salt, 22·78 per cent. of starch was converted into sugar, while without it 25·20 per cent. of starch was converted.

Morphine sulphate.

With this alkaloid O. Nasse* has experimented, using, however, the acetate. He found that the presence of 0·1 per cent. of the salt caused a slight increase in the diastatic action of saliva (109 : 100). Our results with the sulphate of morphine are as follows:

Alkaloid salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1245 gram.	0·2532 gram.	22·78 per cent.
0·05 per cent.	0·1415	0·2880	25·92
0·50	0·1605	0·3276	29·48
2·00	0·1428	0·2908	26·17

The stimulating action of the alkaloid salt up to 2·0 per cent. is very apparent.

Quinine sulphate.

With the acetate of this alkaloid, Nasse found, by the use of 0·1 per cent., an increase in the starch-converting power of the saliva (115 : 100). With the sulphate we obtained the following results:

Alkaloid salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1358 gram.	0·2768 gram.	24·91 per cent.
0·05 per cent.	0·1475	0·3004	27·03
0·50	0·1355	0·2760	24·84
2·00	0·0981	0·1996	17·96

* Pflüger's Archiv, vol. xi, p. 161.

In accord with Nasse's result we see that 0·05 per cent. increases the amylolytic action of the ferment. This we verified by an additional experiment which led to a like result, although not showing so great a difference as the preceding one; thus, while the saliva alone converted 23·72 per cent. starch into reducing bodies, the presence of 0·05 per cent. of quinine sulphate led to the conversion of 24·58 per cent. of starch. Voit, as quoted by v. Boeck,* has stated that quinine is without influence on the ferment of saliva.

Cinchonine sulphate.

With this alkaloid, previous experiments have not to our knowledge been tried. Our results are as follows:

Alkaloid salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1358 gram.	0·2768 gram.	24·91 per cent.
0·05 per cent.	0·1452	0·2960	26·64
0·50	0·1455	0·2964	26·67
2·00	0·1440	0·2936	26·42

Cinchonidine sulphate.

This alkaloid, like the cinchonine, shows a steady accelerating action on the ferment.

Alkaloid salt.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1358 gram.	0·2768 gram.	24·91 per cent.
0·05 per cent.	0·1505	0·3068	27·61
0·50	0·1460	0·2976	26·78
1·75	0·1498	0·3056	27·50

The cinchona group of alkaloids thus show throughout an accelerating influence on amylolytic action, most pronounced in the case of cinchonidine. These alkaloids have long been known to prevent putrefaction and to check alcoholic fermentation and Binz† has demonstrated that this antiseptic action, in the case of quinine at least, is due to the poisonous influence exerted by the latter upon the fungi which are the immediate cause of the putrefactive changes. Couzen, moreover, has shown that the action of cinchonine on infusoria and on fermentation is similar to that of quinine, but weaker. Hence there is no similarity of action whatever, on the two kinds of ferments.

* Zeitschrift für Biologie, vol. vii, p. 428.

† Virchow's Archiv, vol. xlvi, 1869, p. 68.

Atropine sulphate.

With this alkaloid we obtained the following results:

Alkaloid salt.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1485 gram.	0·3028 gram.	27·25 per cent.
0·025 per cent.	0·1460	0·2976	26·78
0·050	0·1410	0·2872	25·62
0·200	0·1530	0·3124	28·11
0·500	0·1407	0·2864	25·77
1·000	0·1475	0·3004	27·03
2·000	0·1245	0·2532	22·78

The main action of the smaller percentages of this alkaloid seems to be a slightly hindering one, although there are one or two irregularities in the results which are not readily explainable. In the presence of 2·0 per cent. of atropine sulphate there is a decided diminution in amylolytic action.

In connection with this alkaloid we have to note some recent experiments of Stolnikow* of St. Petersburg. This investigator, producing artificial fever in dogs by the injection of putrid matter into the blood found, first, that the salivary and pancreatic secretions were for a time increased in amount and then rapidly diminished and finally entirely ceased. This latter action of the septic poison, Stolnikow found to be very persistent and he moreover states that in physiological action the septic poison resembles atropine. Furthermore that artificial fever, produced as described, exercises a decided influence on the *content of ferments* in the pancreatic gland; that in fevers of short duration (2–10 hours) the extract of this gland has a more energetic ferment action than the normal extract, while in fevers of long duration the corresponding extract has a much weaker action. Overlooking now the physiological explanation suggested for these facts we come to the chemical one, viz: that the septic poison possibly exerts either a destructive or hindering influence on the ferment or its action. In support of this view, Stolnikow found that large quantities of the poison did weaken the amylolytic and proteolytic action of extracts from the pancreatic gland, although small quantities of the septic ferment were without action. Likewise, Stolnikow states that small quantities of atropine sulphate are without action on a glycerine extract of the pancreas, but by adding to 10 c. c. of a glycerine extract, 5 c. c. of a 3·0 per cent. atropine sulphate solution and allowing the mixture to stand at the ordinary temperature for 10 hours, then on

* Beiträge zur Lehre von der Function des Pancreas im Fieber. Virchow's Archiv, vol. xc, p. 389, 1882.

testing the amylolytic power of the ferment its action was found to be much weaker than the control. From this fact, Stolnikow considers that the septic poison acts upon the ferment outside the body in a manner similar to atropine.

Now it is obvious, in view of the extreme susceptibility of the ferments of the saliva and pancreas to the action of acids and alkalies, that the atropine solution must be perfectly neutral. Several specimens of atropine sulphate that we have examined, have had a slight acid reaction.

In view of the apparent identity of the amylolytic ferments of the salivary and pancreatic secretions we have repeated in principle Stolnikow's experiment with human saliva, using *perfectly neutral* atropine sulphate.

To 10 c. c. of the dilute, neutral, saliva hitherto used, 0·3 gram of pure atropine sulphate was added (=3·0 per cent. of the alkaloid salt, while Stolnikow's mixture contained but 1·0 per cent.) and the solution allowed to stand for 18 hours at the Laboratory temperature. On now being added to the starch solution, diluted up to 100 c. c. [0·3 per cent. atropine sulphate] and placed at 40° C. for 30 minutes, the starch paste quickly became clear and it was found on examination that 29·16 per cent. of starch had been converted, while the control, in the presence of 0·2 per cent. of atropine sulphate, showed a conversion of 28·11 per cent. of the starch. Hence there had been no *destruction* of the salivary ferment by even 3·0 per cent. of pure atropine sulphate, although as our previous experiments show very much smaller percentages may, by their presence, *hinder the action of the ferment.*

Strychnine sulphate and brucine sulphate.

O. Nasse has previously studied the influence of 0·1 per cent. strychnine acetate on the diastatic action of saliva and has noted a slight increase in amylolytic action in the presence of the strychnine [109: 100]. Our results with the two alkaloids are as follows:

Alkaloid.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1485 gram.	0·3028 gram.	27·25 per cent.
Strychnine sulphate.			
0·050 per cent.	0·1444	0·2936	26·42
0·250	0·1448	0·2936	26·42
0·500	0·1462	0·2976	26·78
Brucine sulphate.			
0·050 per cent.	0·1503	0·3060	27·54
0·500	0·1524	0·3100	27·90
1·000	0·1505	0·3060	27·54

With the brucine salt a slightly increased action is noticed in all three of the experiments; while with strychnine a constant diminution in amylolytic action is to be seen.

In this connection it is to be remembered, that a trace of free acid in the alkaloid salts would introduce an appreciable error into the results, and therefore all of the alkaloid salts experimented with, were especially purified for this purpose, any adhering acid being removed by repeated crystallization, etc.

The following table shows the relative acceleration and retardation of the various salts (the percentages more generally used) compared with their controls expressed as 100.

Table showing relative amylolytic action.

	0·0008 p. c.	0·0005 p. c.	0·001 p. c.	0·002 p. c.	0·005 p. c.	0·010 p. c.	0·025 p. c.	0·05 p. c.	0·1 p. c.	0·5 p. c.	1·0 p. c.	2·0 p. c.	5·0 p. c.
$HgCl_2$	94·3	50·8	38·2	16·7	0								
$HgBr_2$		88·9	59·6	27·3									
HgI_2			97·1	91·2									
$Hg(CN)_2$		106·2	111·7	95·9				77·6					
$CuSO_4+5H_2O$		84·0		31·3		15·4	0						
$Pb(C_2H_3O_2)_2+3H_2O$	100·7	100·3	100·3	97·7	85·2	85·7		99·5	98·6	88·9	78·5		28·5
As_2O_3	102·2	104·4	100·0	106·6	94·2								
H_3AsO_4		100·5	93·0		0								
$(NH_4)_3AsO_4$		106·2	107·0		109·6		111·4	85·8	66·6	10·5	0		
$K(SbO)C_4H_4O_6$			104·5		107·9	114·5		114·5	120·2	168·3	101·6	79·4	30·9
$SnCl_2$	107·5		0										
$ZnSO_4+7H_2O$	99·7	101·3	98·5	96·3	90·9	84·5		52·8	47·5	0			
Fe_2Cl_6		91·5		25·5		6·61	0						
$FeSO_4+7H_2O$		83·2		106·3		109·8							
$K_2Mn_2O_8$					68·5		0						
$MgSO_4+7H_2O$							108·5			35·1			
KCN		86·9	72·2										
$K_4Fe(CN)_6+3H_2O$							105·7		97·0				
$K_6Fe_2(CN)_{12}$							107·0		91·3				
KNO_3										100·9	96·6		92·9
$KClO_3$										104·8	106·0		80·5
KBr										105·6			86·5
KI										104·7			98·7
NaCl										105·5	103·3	103·4	98·2
$Na_2B_4O_7+10H_2O$								0					
$(Mo)_2 . H_2SO_4+5H_2O$								113·7		129·4		114·9	
$(Q)_2 . H_2SO_4+7H_2O$								108·5		99·7		72·1	
$(Ci)_2 . H_2SO_4+2H_2O$								106·9		107·0		106·6	
$(Ci\ dine)_2 . H_2SO_4+3H_2O$								110·8		107·5			
$(At)_2 . H_2SO_4$							98.2	94·0		94·5	99·1	83·5	
$(Sr)_2 . H_2SO_4+6H_2O$								96·9		98·2			
$(Br)_2 . H_2SO_4+H_2O$								101·0		102·4	101·0		

Influence of gases on the amylolytic action of saliva.

The well known analysis by Pflüger* of the gases of the submaxillary saliva have shown the presence of both oxygen and carbonic acid in this secretion; oxygen to the extent of 0·6 vol.-per cent. and carbonic acid, by pump extraction, 22·5 vol.-per cent. It is, moreover, a well known fact that as the saliva flows into the mouth and becomes mixed with the food during mastication much air is absorbed. Do these three gases exert any influence on the amylolytic action of the ferment with which they are so constantly in contact?

Again, the amylolytic ferment of the pancreatic secretion, so near akin, it not identical with the salivary ferment, is subjected to the influence of the reducing gases of the intestinal canal, among which hydrogen may be present to the extent of 22·0† vol.-per cent. and hydrogen sulphide in traces. What likewise is the effect of these two gases on amylolytic action?

The experiments were conducted as follows: 90 c. c. of diluted starch paste were placed in small, partially stoppered flasks and a stream of the gas allowed to pass through, until the fluid was thought to be saturated, then 10 c. c. of dilute saliva were added and the gas allowed to bubble through the solution for 30 minutes when the mixtures were boiled and the reducing bodies determined. Following are the results:

Gases.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0	0·1319 gram.	0·2684 gram.	24·15 per cent.
Air	0·1365	0·2780	25·02
Oxygen	0·1511	0·3080	27·72
Carbonic acid	0·1537	0·3136	28·22
Hydrogen sulphide	0·1377	0·2804	25.23
Hydrogen	0·1248	0·2540	22·86

It is interesting to see that air, oxygen and carbonic acid all stimulate and approximately in proportion to the extent in which they are present in the natural secretion, while of the reducing gases hydrogen retards and hydrogen sulphide stimulates.

The following table shows the relative acceleration and retardation of the several gases, compared with the control, expressed as 100.

Air	103·6
Oxygen	114·7
Carbonic acid	116·8
Hydrogen sulphide	104.4
Hydrogen	94·6

* Physiologische Chemie, Hoppe-Seyler, p. 192.

† Maly in Hermann's Handbuch der Physiologie, vol. v, p. 25.

In accord with our results, Detmer* has found that the presence of carbonic acid invariably increases the amylolytic action of the diastase of malt. The same fact was previously observed by Baswitz.† O. Nasse,‡ however, has stated that the activity of ptyaline in human mixed saliva is not materially affected by oxygen, hydrogen or air. With carbonic acid, however, he noticed acceleration in amylolytic action.

Nature of the action of the metallic and other salts.

In what manner do the metallic and other salts act when they, by their presence, retard or completely stop the amylolytic action of saliva? Is it a process of gradual or sudden destruction of the ferment, or does the metallic salt combine with the ferment, forming a compound incapable of ferment action? or again, is the ferment mechanically thrown down with the precipitate of albumin or globulin produced by the addition of the metallic salt to saliva, or lastly does the salt by its mere presence introduce a condition unfavorable to the action of the ferment? All of these questions are interesting ones, and possibly all of them might be answered in the affirmative and be correct for some one or more of the substances experimented with.

It is obvious that the presence of 10 or 20 per cent. of such a salt as sodium chloride or potassium nitrate in a digestive mixture might retard the action of the ferment, since solutions so saturated, even with the products of digestion, do not admit of vigorous ferment action. But the larger number of metallic salts decidedly retard amylolytic action when present to the extent of only a few thousandths of one per cent., consequently their action must be of an entirely different nature. A number of these salts, such as mercuric chloride, are well known precipitants of albumin, but the saliva being so greatly diluted, in great part for this very reason, cannot yield sufficient precipitate with the mercury salt to mechanically precipitate the ferment. As a matter of fact, when the mercuric chloride solution is added to the diluted saliva, a very faint turbidity only, is produced. If now, some of the small percentages of mercuric chloride are added to the starch solution and then *larger quantities of saliva*, thus giving a larger amount of ferment together with a larger amount of accompanying albumin and globulin, what would

* Zeitschrift für physiol. Chemie, vol. vii, p. 3.

† Berichte d. deutsch. chem. Gesell., vol. xi, p. 1443.

‡ Pflüger's Archiv, vol. xv, p. 471–481.

be the effect on the amylolytic action of the ferment? Might we not expect, knowing that albumin and mercuric chloride readily combine, that the proteid matter present in the saliva, would serve as a shield to protect the ferment from the action of the mercury or other similar metallic salt? At the same time it might be supposed that, the ferment being left intact, any mercury-albumin compound formed might retard or destroy the ferment, though less energetically than the metallic salt alone.

In an attempt to throw some light upon these points the following experiments were tried:

Action of mercuric chloride in the presence of larger amounts of ferment and proteid matter.

a. with 10 c. c. of original saliva.

$HgCl_2$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1772 gram.	0·3624 gram.	32·61 per cent.
0·0005 per cent.	0·1735	0·3548	31·93
0·0010	0·1695	0·3464	31·16

b. with 5 c. c. of original saliva.

0	0·1720 gram.	0·3516 gram.	31·64 per cent.
0·0005 per cent.	0·1340	0·2728	24·55

Comparing these results with those previously obtained with the same percentages of mercuric chloride, but with 2 c. c. of original saliva, we have:

$HgCl_2$.	2 c. c. saliva.	5 c. c. saliva.	10 c. c. saliva.
0	44·28 per cent.	31·64 per cent.	32·61 per cent.
0·0005 per cent.	23·40	24·55	31·93
0·001	16·92	----	31·16

The intensity of action of the mercuric chloride, say 0·0005 per cent. in the three cases, varies greatly; thus with 2 c. c. of saliva the difference in the percentage of starch converted, between the control and the 0·0005 per cent. is 20·88, while with 5 c. c. of saliva the difference is 7·09 and with 10 c. c. of saliva only 0·68. Obviously then, the action of a given percentage of mercuric chloride can be considered as constant only for a given mixture or under definite conditions. Moreover, it would appear (in the 10 c. c.) that either the albuminous matter of the saliva has combined with all of the mercury, leaving the ferment free to act in a normal manner, except so far as it is impeded by the mercury-albumin compound, or else that only a small proportion of the ferment has been chemically precipitated, leaving an amount sufficient for energetic amylolytic action, since, as is well

known, increase or decrease in the amount of ferment is not always followed by a proportionate change in the amount of reducing bodies formed. Of these two views the former is by far the most probable. Certainly the ferment is not mechanically precipitated by the formation of a mercury-albumin precipitate; if such were the case with 10 c. c. of saliva and 0·001 per cent. of mercuric chloride, decided retardation ought to have been observed.

Action of cupric sulphate in the presence of larger amounts of ferment and proteid matter.

a. with 10 c. c. saliva.

$CuSO_4+5H_2O$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1830 gram.	0·3748 gram.	33·73 per cent.
0·0005 per cent.	0·1775	0·3628	32·65
		Difference,	1·08

b. with 5 c. c. saliva.

0	0·1745 gram.	0·3568 gram.	32·11 per cent.
0·0005 per cent.	0·1640	0·3352	30·16
		Difference,	1·95

c. with 2 c. c. saliva.

0	0·1645 gram.	0·3360 gram.	30·24 per cent.
0·0005 per cent.	0·1140	0·2320	20·88
		Difference,	9·36

Action of zinc sulphate in the presence of larger amounts of ferment and proteid matter.

a. with 10 c. c. saliva.

$ZnSO_4+7H_2O$.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·1830 gram.	0·3748 gram.	33·73 per cent.
0·05 per cent.	0·1737	0·3552	31·96
		Difference,	1·77

b. with 5 c. c. saliva.

0	0·1745 gram.	0·3568 gram.	32·11 per cent.
0·05 per cent.	0·1610	0·3288	29·59
		Difference,	2·52

c. with 2 c. c. saliva.

0	0·1645 gram.	0·3360 gram.	30·24 per cent.
0·05 per cent.	0·1320	0·2688	24.19
		Difference,	6·05

Glancing at the *differences* in these two series of experiments, we see that they accord with what was observed in the case of mercuric chloride, viz: that a given percentage of the metallic salt will produce a constant result only under definite conditions; increasing the proportion of albuminous matter diminishes, as in the case of the mercury salt, although not so greatly, the retarding action of the salt.

Evidently, the metallic salts do not act upon the ferment by their mere presence, for if such were the case the mere combination of the salt with the albumin present, would not so materially affect the result. If, on the other hand, they do act by combining with the ferment, forming it may be an insoluble compound or one incapable of ferment action, it is fair to presume that the combination would take place immediately upon mixing the two or very soon thereafter, and thus we should expect that the length of time the two stood in contact after the first few minutes, would have no effect on the amylolytic power of the mixture, while a gradual *destructive action* would be manifested by a gradual decrease of amylolytic power. With a view to testing this point we have tried the following experiment. Three mixtures were prepared as follows:

	A.	*B.*	*C.*
Saliva	2 c. c.	2 c. c.	2 c. c.
H_2O	8	7	8
$HgCl_2$ sol.	0	1	2
	10	10	10
Per cent $HgCl_2$	0	0·005	0·010

These were placed in a bath and warmed at 40° C. for 18 hours, after which 1 c. c. of the same mercuric chloride solution was added to A and then starch and water added to all three, making the volume in each case up to 100 c. c. The mixtures were then warmed at 40° C. for thirty minutes to test the activity of the ferment; *A* containing now 0·0005 per cent. mercuric chloride, *B* the same percentage and *C* 0·001 per cent.. In *A*, 19·8 per cent. of the starch was converted into reducing bodies, while in *B* and *C* there was no amylolytic action whatever. Thus by the previous action, for this length of time, of 0·005 per cent. mercuric chloride, the ferment was rendered incapable, on subsequent dilution, of exerting any diastatic action whatever.

Again, in a similar manner it was found that by warming the saliva for thirty minutes at 40° C. with 0·005 per cent. mercuric chloride and then adding starch paste and diluting to 100 c. c. so

that the percentage of mercuric chloride was 0·0005, only 2·0 per cent. of the starch was converted, while the same quantity of saliva, in the presence of the same amount of mercury salt (0·0005 per cent.) converted 19·69 per cent. of starch.

Working with larger amounts of saliva, the following results were obtained:

	A.	*B.*	*C.*
Saliva	10 c. c.	10 c. c.	10 c. c.
H_2O	16	15	15
$HgCl_2$ sol.	0	1	1
	26	26	26
Per cent. $HgCl_2$	0	0·002	0·002

B was warmed at 40° C. for 15 minutes and *C* for 30 minutes; then 1 c. c. of the mercuric chloride solution was added to *A*, and all three diluted and mixed with starch paste. The three solutions were now exactly alike; all contained the same percentage of mercury salt (0·0005 per cent.) but *B* and *C* had been previously warmed with the salt for 15 and 30 minutes respectively. *A*, converted 31·32 per cent. of the starch, *B* 29·48 per cent. and *C* 27·97 per cent. Here we have what appears to be a gradual decrease in amylolytic power, but it does not seem sufficiently pronounced to account for the action of the mercury salt. It would appear rather, in this instance, as if the mercuric chloride exercised a selective action, combining with the proteid matter of the saliva, leaving the ferment free; but the mercury-proteid compound, being apparently possessed of some destructive action, exerts its influence, and thus the gradual decrease of amylolytic power noticed in *B* and *C*.

In the previous experiments, on the other hand, where *free* mercuric chloride is present, there not being sufficient albumin to combine with all of the mercury, there is apparently destructive action.

Experiments of like nature as the preceding, tried with cupric sulphate, gave the following results:

	A.	*B.*	*C.*	*D.*
Saliva	2 c. c.	2 c. c.	2 c. c.	2 c. c.
H_2O	18	17·8	17·8	17·8
$CuSO_4$ sol.	0	0·2	0·2	0·2
	20	20·0	20·0	20·0
Per cent. $CuSO_4$	0	0·0005	0·0005	0·0005

B was warmed at 40° C. for 15 minutes, *C* for 30 minutes and *D* for 1 hour; 0·2 c. c. of the cupric sulphate solution was then added to *A* and lastly starch paste and water to 100 c. c. The amylolytic

power of the four mixtures, expressed in the percentage of starch converted, was as follows:

A.	*B.*	*C.*	*D.*
28·72	22·35	23·04	20·23

With zinc sulphate, somewhat similar results were obtained:

	A.	*B.*	*C.*
Saliva	2 c. c.	2 c. c.	2 c. c.
H_2O	18	16	16
$ZnSO_4$ sol.	0	2	2
	20	20	20
Per cent. $ZnSO_4$	0	0·05	0·05

B was warmed at 40° C. for 30 minutes and *C* for 1 hour; then 2 c. c. of the zinc sulphate solution were added to *A*, and all three mixed with starch paste and water to 100 c. c. Each now contained 0·01 per cent. zinc sulphate and all three were then warmed at 40° C. for 30 minutes, to determine the activity of the ferment. *A*, converted 22·24 per cent. of the starch, *B* 11·88 per cent. and *C* 10·98 per cent.

These experiments would therefore indicate, on the part of the metallic salts experimented with, a destructive action towards the ferment, though loss of amylolytic power under the conditions of the experiments might also be due to more complete precipitation of the ferment in the more concentrated solution and under longer exposure to a temperature of 40° C. At the same time it is to be noticed, that any metallic-proteid compound formed with the above salts, has a far less destructive or retarding action than the free salt. Of these, the destructive action of mercuric chloride is most pronounced.

Potassium permanganate acts, doubtless, by direct destruction of the ferment through oxidation, while many of the alkali and alkali-earth salts produce their retarding effects by simple clogging of the digestive fluid; but the fact that 0·5 per cent. of one salt, as potassium antimony tartrate, for example, increases the amount of starch converted 68 per cent., and 0·5 per cent. of another salt, as magnesium sulphate, diminishes the amount of starch converted by 65 per cent., plainly indicates that there is something in the presence of these salts, dependent upon chemical constitution, that controls the action of the ferment.

Influence of Various Inorganic and Alkaloid Salts on the Proteolytic Action of Pepsin-hydrochloric Acid. By R. H. Chittenden and S. E. Allen.

Although many experiments have been tried to ascertain the influence of various salts on ferment action since 1870, when Liebig* recorded the statement that the fermentative power of yeast is somewhat increased by a little potassium or sodium chloride, few systematic experiments, with a large variety of salts, have been made with the ferment of the gastric juice.

Alex. Schmidt† in 1876 studied the influence of sodium chloride on the digestive action of pepsin and hydrochloric acid. Wolberg‡ in 1880 studied, with the same ferment, the action of ammonium, potassium and sodium salts of nitric, hydrochloric and sulphuric acids and also the action of several alkaloids. Wernitz§ and also Petit‖ have studied the action of several metallic salts. Still later, Pfeiffer¶ has examined the influence of several alkali and alkali-earth salts on the digestive action of pepsin as well as of other ferments. Isolated experiments with single salts have likewise been recorded; these will be noticed later on.

It is thus seen that almost all work in this direction has been done with salts of the alkali and alkali-earth metals. No systematic attempt has been made to ascertain the influence on gastric digestion of the large number of metallic salts, in common use as poisons or therapeutic agents. With the exception of a few isolated cases, no accurate data are recorded bearing on this question. Observation has led to the belief that certain metallic salts interfere with digestion in the stomach, but few quantitative results are recorded to show the truth of such a belief.

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* Ueber Gährung, Quelle der Muskelkraft und Ernährung. Separatabdruck aus den Annalen der Chemie u. Pharmacie, 1870, p. 61.

† Pflüger's Archiv, vol. xiii, p. 97. Ueber die Beziehung des Kochsalzes zu einigen thierischen Fermentationsprocessen.

‡ Pflüger's Archiv, vol. xxii, p. 291. Ueber den Einfluss einiger Salze und Alkaloiden auf die Verdauung.

§ Quoted by Brunton. Pharmacology, p. 85–86.

‖ Études sur les ferments digestifs. Abstract in Jahresbericht für Thierchemie, 1880, p. 309.

¶ Ueber den Einfluss einige Salze auf verschiedene künstliche Verdauungsvorgänge. Abstract in Centralbl. med. Wiss., 1885, p. 328.

Our aim has been, therefore, to study more particularly the comparative influence on gastric digestion of various percentages of those salts, well known as poisons or therapeutic agents, which have hitherto been overlooked or but imperfectly studied. At the same time in order to make the work more complete, we have studied somewhat, the action of the alkali salts, experimented with by other observers.

Method employed.

The experiments were conducted in series, in which one of each series served as a control for comparison. The artificial gastric juice employed, was made from 0·2 per cent. hydrochloric acid and a glycerine extract of pepsin, in the proportion of 10 c. c. of the latter to 1 litre of the former. The volume of each digestive mixture was 50 c. c.; made up of 25 c. c. of the above-mentioned gastric juice and 25 c. c. of 0·2 per cent. hydrochloric acid, containing the salt to be experimented with. The material to be digested, consisted of purified and dried blood-fibrin, prepared by thorough washing with water, extraction with cold and boiling alcohol and lastly with ether. It was then ground to a coarse powder and dried at 100–110° C. 1 gram of the fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for two hours, then filtered upon weighed filters by the aid of pumps, the residue washed thoroughly with water, lastly with alcohol, and finally dried at 100–110° C. until of constant weight (48 hours). The amount of fibrin digested or dissolved, is a measure of the proteolytic action.

Cupric sulphate.

With this salt two series of experiments were made; one to ascertain the influence of small quantities, the other to show the effects of larger amounts of the substance.

$CuSO_4+5H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2854 gram.	71·46 per cent.	100·0
0·001 per cent.	0·2508	74·92	104·8
0·005	0·2650	73·50	102·8
0·010	0·3067	69·33	97·0
0·025	0·3845	61·55	86·1
0·050	0·3877	61·23	85·6
0	0·2352	76·48	100·0
0·1	0·5315	46·85	61·2
0·3	0·7585	24·15	31·5
0·5	0·7976	20·24	26·4
0·8	0·8214	17·86	23·3
1·5	0·8480	15·20	19·8

The action of the salt is very marked; with even 0·010 per cent. there is a diminution in proteolytic action amounting to 3·0 per cent., while in the presence of 0·5 per cent. of the salt, there is retardation to the amount of nearly 75 per cent. The copper salt prevents almost entirely the swelling of the fibrin and doubtless its retarding action is due in part to this fact.

Lead acetate.

In view of the frequent cases of chronic poisoning with lead salts, the influence of the acetate on gastric digestion, seems especially interesting. The results, moreover, show decided action on the part of the salt; with small fractions of a per cent. pronounced increase in proteolytic action is to be noticed, while beyond 0·5 per cent. there is sudden and almost complete cessation of ferment action. In this respect, the salt acts very differently from the copper salt, with which a more gradual diminution is observed. The two largest percentages of the lead salt prevented entirely the swelling of the fibrin.

$Pb(C_2H_3O_2)_2+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1936 gram.	80·64 per cent.	100·0
0·001 per cent.	0·1592	84·08	104·2
0·005	0·1892	81·08	100·5
0·010	0·1781	82·19	101·9
0·025	0·1691	83·09	103·0
0	0·2140	78·60	100·0
0·1	0·2310	76·90	97·8
0·3	0·4523	54·77	69·6
0·5	0·7419	25.81	32·8
0·8	0·9779	2·21	2·8
1·5	0·9938	0·62	0·7

Mercuric chloride.

This salt, which showed such a marked action on the amylolytic ferment of the saliva, causes a like diminution of proteolytic action in the case of pepsin; even with 0·001 per cent. there is retardation to the extent of over 6 per cent., calling the action of the control 100.

Petit* very erroneously states that mercuric chloride up to 0·4 per cent. does not hinder the action of pepsin.

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* Études sur les ferments digestifs. Abstract in Jahresbericht für Thierchemie, 1880, p. 309.

$HgCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3759 gram.	62·41 per cent.	100·0
0·001 per cent.	0·4140	58·60	93·8
0·005	0·4210	57·90	92·7
0	0·1307	86·93	100·0
0·1	0·4765	52·35	60·2
0·5	0·9007	9·93	11·4
1·0	1·0495	0	0

M. Marle* has previously experimented with mercuric chloride and has likewise found that small quantities of the salt exercise a retarding action upon gastric digestion; that as the percentage of corrosive sublimate is increased, the retarding action is correspondingly increased, although this effect is diminished up to a certain point, by increasing the strength of the digestive mixture. Marle considers that this action of mercuric chloride does not depend upon decomposition of the ferment nor upon a contraction of the albuminous matter, but rather that the salt in an acid solution enters into a chemical combination with the proteid matter and the latter is thus rendered impervious to the digestive action of the ferment.

In support of this view we offer the fact that fibrin introduced into an acid solution of pepsin in the presence of 1 per cent. of mercuric chloride, increases in weight; in the experiment given above to the extent of 49·5 milligrams. This would clearly indicate a combination of the two. Moreover, that mercuric chloride does not act by destroying the ferment we have ample proof, as the following experiment shows:

	A.	*B.*	*C.*
H_2O sol. glycerine pepsin	5 c. c.	5 c. c.	5 c. c.
HCl (0·2 per cent.)	20	20	0
$HgCl_2$	0	0·025 gram.	0·025 gram.
H_2O	0	0	20 c. c.
	25	25 c. c.	25
Per cent. $HgCl_2$	0	0·1	0·1

These three mixtures were warmed at 40° C. for 24 hours; then to *A* was added 0·025 gram $HgCl_2$ dissolved in 25 c. c. 0·2 per cent. HCl, to *B* 25 c. c. 0·2 per cent. HCl and to *C* 25 c. c. 0·4 per cent. HCl. The three solutions were now exactly alike; in *B*, however, the ferment had been exposed to the action of 0·1 per cent. $HgCl_2$ in an acid solution for 24 hours, in *C* to the action of the same percentage of the mercury salt in an aqueous solution, while *A* served as

* Abstract in Jahresbericht für Thierchemie, 1875, p. 168.

a control. 1 gram of fibrin was added to each of the mixtures, which were then placed at 40° C. for 2 hours. *B* and *C* digested the same amount of fibrin as *A*, consequently the mercuric chloride could have exerted no destructive action whatever on the ferment.

Wassilieff,* in Hoppe-Seyler's laboratory, found by comparative experiments that mercurous chloride (calomel) has no effect on the proteolytic action of pepsin.

Mercuric bromide, Mercuric iodide and Mercuric cyanide.

These three salts of mercury were experimented with, only so far as to compare the action of small quantities, with the action of like quantities of mercuric chloride. In using the bromide and iodide it was necessary, on account of their insolubility, to dissolve them with the aid of an equal weight of sodium chloride, consequently these two salts of mercury were doubtless present in the digestive mixtures, in part at least, as double salts. Marle, however, found that the action of mercuric chloride with small quantities of sodium chloride was not different from that of mercuric chloride alone, and doubtless the same is true of the iodide and bromide of mercury. Following are the results we obtained:

Mercury salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3590 gram.	64·10 per cent.	100·0
$HgBr_2$			
0·005 per cent.	0·3731	62·69	97·8
0·025	0·3980	60·20	93·9
HgI_2			
0·005	0·3114	68·86	107·4
0·025	0·3904	60·96	95·1
$Hg(CN)_2$			
0·005	0·3105	68·95	107·5
0·025	0·3985	60·15	93·8
0·100	0·3183	68·17	106·3

From these it is evident that mercuric bromide is less vigorous in its hindering action than mercuric chloride; the iodide still less so, while mercuric cyanide, in similar percentages, appears to cause an increase in proteolytic action. The iodide, likewise, in the smallest percentage experimented with, causes increased proteolytic action. None of these salts then, approach mercuric chloride in the intensity of its hindering action on gastric digestion.

* Ueber die Wirkung des Calomel auf Gährungsprozesse und das Leben von Mikroorganismen. Zeitschrift f. Physiologische Chemie, vol. vi, 113.

Stannous chloride.

This salt shows marked action in retarding gastric digestion; its retarding effect increasing directly with the amount of stannous chloride added.

$SnCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2576 gram.	74·24 per cent.	100·0
0·025 per cent.	0·2728	72·72	97·5
0·1	0·4826	51·74	69·6
0·5	0·7332	26·68	35·9
1·0	0·8155	18·45	24·8
2·0	0·9010	9·90	13·3

Arsenious oxide.

This substance might naturally be expected, in view of its well known antiseptic properties, to hinder proteolytic action, more or less. It is known to hinder putrefaction and to prevent also the fermentative action of yeast. Contrary to our expectations, however, the action of arsenious oxide, so far as it is to be seen, is an accelerating one, causing increased proteolytic action. The following results were obtained:

As_2O_3.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2111 gram.	78·89 per cent.	100·0
0·05 per cent.	0·1872	81·28	103·0
0·1	0·2160	78·40	99·3
0·2	0·1900	81·00	102·6
0·5	0·1707	82·93	105·1

The stimulating action is slight, still it is plainly recognizable. Drs. Schäfer and Böhm* have previously studied the action of arsenious acid on the digestion of albumin by artificial gastric juice, and they came to the conclusion, using 0·02 and 0·04 gram As_2O_3 respectively, in 34 c. c. of fluid containing egg-albumin, that arsenious oxide is without influence on the decomposition of albumin by the gastric juice ferment. Our results, though not so large in number as theirs, would indicate a slight accelerating action.

Arsenic is known, when administered in small, repeated doses, to act as a tonic; the history of arsenic-eating, indicates that the substance has some positive tonic influence over nutrition, and Dr.

* Jahresbericht für Theirchemie, 1872, p. 363. Ueber den Einfluss des Arsens auf die Wirkung der ungeformten Fermente.

Wood* states, "there is much reason for believing that it acts largely as a direct stimulant to nutrition." The results obtained in our experiments certainly accord with this statement.

Arsenic acid.

The experiments tried with arsenic acid, tend to confirm the accelerating action noticed with arsenious oxide. In the first series of experiments the following results were obtained:

H_3AsO_4.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2696 gram.	73·04 per cent.	100·0
0·2 per cent.	0·2614	73·86	101·1
0·5	0·1514	84·86	116·1
2·0	0·2583	74·17	101·5
5·0	0·3915	60·85	83·3

The accelerating action is here so very pronounced, that a second series of experiments was undertaken by way of confirmation. These give in a general way the same results, although with 0·5 per cent. the stimulating action is not so pronounced as in the first experiment.

These two series of experiments illustrate another point, which it is well to mention here, namely: that definite percentages of any particular substance do not invariably give precisely the same result, even when compared with their respective controls. They do, however, generally point in the same direction, and although not always giving exactly the same numerical expression, they show clearly the nature and extent of the action.

H_3AsO_4.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2490 gram.	75·10 per cent.	100·0
0·2 per cent.	0·2401	75·99	101·2
0·5	0·2367	76·33	101·6
1·0	0·2335	76·65	102·0
2·0	0·2622	73·78	98·2
5·0	0·3176	68·24	90·8
0	0·1493	85·07	100·0
10·0	0·4207	57·93	68·1

Plainly then, arsenic acid in small percentages does accelerate the proteolytic action of pepsin-hydrochloric acid, while in large percentages (5–10) it causes a diminution in the action of the ferment. Arsenic acid tends to make the fibrin become very gelatinous.

* Therapeutics, Materia Medica and Toxicology, p. 390.

Zinc sulphate.

With this salt, no experiments appear to have been hitherto made. Our results show a decided diminution in proteolytic action, even in the presence of 0·01 per cent. of the salt, while with a few thousandths of one per cent. the figures indicate a slight accelerating action. Three distinct experiments were made as follows:

$ZnSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1744 gram.	82·56 per cent.	100·0
0·001 per cent.	0·1609	83·91	101·6
0·005	0·1617	83·83	101·5
0·010	0·2053	79·46	96·2
0·025	0·2573	74·27	89·9
3·000	0·8400	16·00	19·3
0	0·1630	83·20	100·0
0·1	0·4848	51·52	61·9
0·3	0·7133	28·67	34·4
0·5	0·7382	26·18	31·4
0·8	0·7671	23·29	27·9
1·5	0·8202	17·98	21·6
0	0·1493	85·07	100·0
1·0	0·7683	23·17	27·2

A glance at these results, shows plainly a gradual decrease in proteolytic activity.

It is to be noticed that in the presence of the larger percentages of these metallic salts, the fibrin does not swell up in the 0·2 per cent. acid.

Manganous chloride.

In small fractions of one per cent. this salt gave such irregular results that it is doubtful if they can be relied upon as expressing any particular action. With 0·3 per cent. the retarding action of the manganese salt commences to be very pronounced. Following are the results:

$MnCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·1923 gram.	80·77 per cent.	100·0
0·001 per cent.	0·2022	79·78	98·7
0·010	0·1815	81·85	101·3
0·025	0·2066	79·34	98·2
0·050	0·1855	81·45	100·8
0	0·1880	81·20	100·0
0·3	0·3687	63·13	77·7
0·8	0·6438	35·62	43·8
1·5	0·6612	33·88	41·7
3·0	0·7400	26·00	32·0

Ferrous sulphate and Ferric chloride.

The salts of iron, doubtless on account of their physiological importance and their great therapeutic value, have been experimented with by several observers. It has been a prevalent opinion that iron salts tend to produce disturbances in gastric digestion. Petit,* however, states as a result of experiment, that preparations of iron, in small quantities, do not hinder the action of pepsin, but in large quantities they retard the action of the ferment, doing so according to Petit, by the hydrochloric acid of the gastric juice displacing the acid of the iron salt, thus forcing the pepsin to act with a less energetic acid. Düsterhoff,† dealing with the same question, came to the conclusion that iron salts of the organic acids, exercise the greatest retarding effect on pepsin digestion, and moreover, that ferrous salts are better adapted to the organism than ferric salts. Düsterhoff also concludes that while the retarding action of iron salts is doubtless due, in part, to the setting-free of the acid of the iron salt by the acid of the gastric juice, there is in addition a specific action of the iron preparation of an unknown nature, prejudicial to digestion. Lastly, Bubnow‡ found that moist ferric hydroxide in small quantities (not weighed) causes a scarcely recognizable diminution in proteolytic action, while the presence of 1 per cent. of ferrous chloride and ferrous sulphate causes marked retardation, as does also an excess of ferric hydroxide. The most intense action was observed on the addition of 5 per cent. of ferrous sulphate. No quantitative results, that is, percentages of albumin digested were, however, obtained.

Our experiments were made only with crystallized ferrous sulphate and ferric chloride. It appears superfluous to try the action of ferric hydroxide, which must necessarily, if in sufficient quantity, neutralize the acid of the gastric juice and thus prevent digestion by withdrawal of the free acid.

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* Quoted by Bubnow in Zeitschrift für physiologische Chemie, vol. vii, p. 316; also abstract by Herter in Jahresbericht für Thierchemie, 1880, p. 309.

† Ueber den Einfluss von Eisenpräparaten auf die Magenverdauung. Jahresbericht für Thierchemie, 1882, p. 257.

‡ Ueber den Einfluss des Eisenoxyhydrats und der Eisenoxydulsalze auf künstliche Magenverdauung und Fäulniss mit Pancreas. Zeitschrift für Physiologische Chemie, vol. vii, p. 315.

$FeSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1835 gram.	81·65	100·0
0·001 per cent.	0·1916	80·84	99·0
0·005	0·2241	77·59	95·0
0·010	0·1895	81·05	99·2
0·025	0·2573	74·27	90·9
0·050	0·2773	72·27	88·5
0	0·1935	80·65	100·0
0·1	0·3467	65·33	81·0
0·3	0·7274	27·26	33·8
0·8	0·8080	19·20	23·8
1·5	0·8447	15·53	19·2

Here, with the ferrous salt, we find pronounced diminution of proteolytic action, commencing even with 0·001 per cent. With ferric chloride, the following results were obtained.

Fe_2Cl_6.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1842 gram.	81·58 per cent.	100·0
0·001 per cent.	0·2111	78·89	96·7
0·005	0·2059	79·41	97·3
0·010	0·2165	78·35	96·0
0·050	0·2332	76·68	93·9
0	0·1961	80·39	100·0
0·3	0·6526	34·74	43·2
0·5	0·8035	19·65	24·4
0·8	0·8794	12·06	15·0
3·0	0·9582	4·18	5·2

A comparison of the two series of results, shows no pronounced and constant difference in the amount of action between the two iron salts; both retard proteolytic action about equally; although with the larger amounts, as with 0·5 per cent. and beyond, ferric chloride appears the most injurious. Comparing the results with those obtained with the manganese salt, which of late has been recommended as a therapeutic agent where iron cannot be taken, we see that the manganese is throughout, far less injurious than the two salts of iron.

As to the manner in which the iron salts produce their retarding effect on proteolytic action, it is evident that it cannot be due to a simple displacement of the acid of the iron salt, by which the pepsin is made to act with a less compatible acid, since ferric chloride acts similarly to the sulphate, in which case there could be no such injurious replacement.

Magnesium sulphate.

Pfeiffer * alone appears to have studied the influence of magnesium sulphate on gastric digestion. He found that retarding action commenced in the presence of 0·24 per cent. of the salt and was very great in the presence of 4·0 per cent. Our results show decided retarding action, even in the presence of 0·005 per cent. of the crystallized salt. At the same time, it is to be remembered throughout, that probably differences in the strength of gastric juice, would cause some variation in the amount of retardation, produced by any given percentage.

$MgSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1081 gram.	89·19 per cent.	100·0
0·005 per cent.	0·1910	80·90	90·7
0·010	0·2330	76·70	86·0
0·050	0·3260	67·40	75·5
0·100	0·4428	55·72	62·3
0	0·2605	73·95	100·0
0·3	0·7551	24·49	33·1
0·5	0·7886	21·14	28·5
0·8	0·8250	17·50	23 7
1·5	0·8891	11·09	15·0
3·0	0·8894	11·06	14·9

It is noticeable here, that while the retarding influence of the salt, becomes more and more pronounced as the percentage is increased, there comes a point (1·5 per cent.) when further addition does not materially influence the action of the ferment.

Potassium permanganate.

This salt, as with the amylolytic ferment of the saliva, shows very energetic action. Its influence is, without doubt, due to rapid oxidation and consequent destruction of the ferment; indeed, the (at first) bright red color of the solution became almost immediately bleached out and the solution, at the same time, completely deprived of proteolytic power. The following results testify to its extreme activity.

$K_2Mn_2O_8$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1951 gram.	80·48 per cent.	100·0
0·005 per cent.	0·8278	17·22	21·3
0·010	0·9949	0·51	0·6

It is thus more active in preventing proteolytic action, in gastric juice of the strength used, than in hindering the development of

* Abstract in Centralbl. med. Wiss., 1885, p. 328.

bacteria; although doubtless, the action of any one percentage is dependent in part, upon the amount of organic matter present. Marcus and Pinet* found that the permanganate in 0·1 per cent. would prevent the development of bacteria and in 1·5 per cent. would kill the fully developed organisms.

Potassium dichromate.

A single experiment with this salt gave the following results; showing a decided retarding action on gastric digestion.

$K_2Cr_2O_7$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2028 gram.	79·72 per cent.	100·0
0·01 per cent.	0·2476	75·24	94·4
0·10	0·6383	36·17	45·3

Potassium cyanide.

Potassium cyanide we found very active in diminishing the digestive power of pepsin; due in great part doubtless, to decomposition of the cyanide by the hydrochloric acid of the gastric juice with conseqent formation of pepsin-hydrocyanic acid. Our first results were as follows:

KCN.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3255 gram.	67·45 per cent.	100·0
0·25 per cent.	0·9687	3·13	4·6
0·50	0·9912	0·88	1·3

Here, the fibrin did not swell at all, indicating the probable absence of free hydrochloric acid, although of course the potassium cyanide might, *per se*, prevent swelling.

With very much smaller percentages of cyanide, we obtained the following results:

KCN.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3098 gram.	69·02 per cent.	100·0
0·005 per cent.	0·4376	56·24	81·5
0·025	0·3750	62 50	90·5

Potassium ferrocyanide.

With this salt, the results are practically the same as with potassium cyanide; almost complete stopping of proteolytic action, even in the presence of small fractions of one per cent.

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* Action de quelques substances sur les bactéries de la putréfaction, Compt. rend. Soc. de Biolog., 1882, p. 718. Abstract in Jahresbericht für Thierchemie, 1882, p. 515.

$K_4Fe(CN)_6+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3717 gram.	62·83 per cent.	100·0
0·05 per cent.	0·5969	40·31	64·1
0·10	0·7922	20·78	33·0
0·25	0·9585	4·15	6·6
0·5	1·0	0	0
0	0·3098	69·02	100·0
0·005	0·3562	64·38	93·3
0·025	0·3471	65·29	94·5

Potassium chlorate and Potassium nitrate.

These two oxidizing agents produce almost exactly the same effect on pepsin-hydrochloric acid digestion; a retarding action directly proportional to the amount of salt present.

$KClO_3$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2683 gram.	73·17 per cent.	100·0
0·3 per cent.	0·4565	54·35	74·3
0·8	0·7019	29·81	40·7
1·5	0·8173	18·27	25·0
3·0	0·8707	12·93	17·6
KNO_3.			
0	0·2870 gram.	71·30 per cent.	100·0
0·3 per cent.	0·5370	46·30	64·9
0·5	0·6247	37·53	52·6
0·8	0·7158	28·42	39·8
1·5	0·8148	18·52	25·9
3·0	0·8907	10·93	15·3

With potassium chlorate no experiments have been previously tried; with potassium nitrate, however, Wolberg* experimenting with quantities varying from 0·5 to 8·0 per cent. found in every instance, diminution in the proteolytic action of his pepsin solution. This, however, amounted to but little, except in the presence of 8 grams (8 per cent.) of the nitrate, where there was a diminution in the amount of fibrin digested, equal to 49·0 per cent. Even with 6 per cent. of the salt, Wolberg found after 24 hours, only a diminution of 6·8 per cent. in the fibrin digested.

In quantity, therefore, our results do not accord at all with Wolberg's, since as the table shows, even 0·3 per cent. of potassium nitrate caused a diminution in the quantity of fibrin digested, amounting to 35·1 per cent., when compared with the control (100); while the pres-

* Pflüger's Archiv, vol. xxii, p. 300. Ueber den Einfluss einiger Salze und Alkaloiden auf die Verdauung.

ence of 3 per cent. of the salt caused a diminution in proteolytic action amounting, in the quantity of fibrin digested, to nearly 85 per cent. The only apparent explanation of this difference in the results [unless due to difference in the amount of ferment] is in the length of time the mixtures were warmed at 40° C.; in Wolberg's 24 hours, in ours 2 hours. This, if the true reason of the difference, would imply on the part of the ferment, ability to gradually overcome the influence of small amounts of the substance and thus eventually to digest an equal quantity of proteid matter. This, however, would in turn imply that the object sought for, viz: the influence of different quantities or percentages of a substance on the action of the ferment is lost sight of. The length of time best adapted to the experiment, is naturally that which will bring out most clearly and decisively all *differences* of action.

Sodium tetraborate (Borax) *and Boracic acid.*

Sternberg's experiments* with both of these substances, have shown that, although possessed of no germicide value, they prevent the multiplication of bacterial organisms and are thus valuable antiseptics.

Wolberg, in experiments made with artificial gastric juice, found that in a 24 hours digestion, 0·5 gram (0·5 per cent.) of borax caused a slight acceleration in proteolytic action (0·4 per cent.), while with 1 per cent. of the salt, retardation occurred to the extent of 23·3 per cent., and in the presence of 4·0 per cent. almost complete stopping of proteolytic action. Our results, however, fail to show any stimulating action on the part of the borate, although retardation is very pronounced.

$Na_2B_4O_7+10H_2O$.	Undigested residue.	Fibrin digested	Relative proteolytic action.
0	0·3610 gram.	63·90 per cent.	100·0
0·05 per cent.	0·3852	61·48	96·2
0·20	0·4080	59·20	92·6
0·5	0·7710	22·90	35·8
1·0	0·9899	1·01	1·5

Doubtless, the retarding action of this salt is due wholly to the liberation of boracic acid and the consequent neutralization of the hydrochloric acid of the gastric juice. Boracic acid itself, offers no obstacle to the proteolytic action of pepsin-hydrochloric acid; on the contrary it increases it, but pepsin-boracic acid has little digestive

* Amer. Jour. Med. Sciences, April, 1883, p. 335.

power. The influence of boracic acid on pepsin-hydrochloric acid is seen from the following experiments:

H_3BO_3.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0 2395 gram.	76·05 per cent.	100·0
0·1 per cent.	0·2232	77·68	102·1
0	0·2049	79·51	100·0
0·5	0·1875	81·25	102·2
3·0	0·1729	82·71	104·2
6·0	0·1445	85·55	107·6

Evidently then, the action of borax consists simply in withdrawing from the pepsin the hydrochloric acid of the gastric juice. The low digestive power of pepsin and boracic acid is shown by the following experiment:

	A.	*B.*	*C.*	*D.*
H_2O sol. pepsin	50 c. c.	50 c. c.	50 c. c.	50 c. c.
HCl 0·2 per cent.	50	0	0	0
H_3BO_3	0	0·2 gram.	0·3 gram.	0·5 gram.
H_2O	0	50 c. c.	50 c. c.	50 c. c.
	100	100	100	100
	0·1 % HCl	0·2 % H_3BO_3	0·3 % H_3BO_3	0·5 % H_3BO_3

To each, was added 1 gram of purified fibrin, after which the mixtures were warmed at 40° C. for 2 hours. Following are the results.

	A.	*B.*	*C.*	*D.*
Wt. of undigested residue	0·1180	0·9615	0·9705	0·9620
Per cent. digested	88·20	3·85	2·95	3·80

Ammonium oxalate.

With this salt the following results were obtained:

$(NH_4)_2C_2O_4+2H_2O$.	Undigested. residue.	Fibrin digested.	Relative proteolytic action.
0	0·3254 gram.	67·46 per cent.	100·0
0·010 per cent.	0·3579	64·20	95·1
0·025	0·3627	63·73	94·6
0·1	0·3920	60·80	90·1
0·5	0·9049	9·51	14·1
0	0·3098	69·02	100·0
1·0	0·9958	0 42	6·6

As to the cause of this retarding action, it is probable, that, as in the case of borax, the oxalic acid of the salt is displaced and the ferment compelled to act with the acid thus liberated. But if we compare the results of the present series with those of the preceding,

we find that, with like percentages of the two salts, ammonium oxalate has far the greater retarding power, and yet it is well known that the ferment acts well when combined with oxalic acid. Moreover, our experiments show further on, that ammonium chloride has no greater retarding power than sodium chloride. Hence, there must be some reason, other than the one mentioned above, to account for all of the retarding action manifested by the oxalate.

Petit* states, that the maximum digestive action of oxalic acid, with 0·2–0·4 per cent. of his pepsin, is attained with 0·5–4·0 per cent. of the acid, according to the amount of pepsin.

The comparative digestive power of pepsin-oxalic acid and pepsin-hydrochloric acid is shown by the following experiments:†

	A.	*B.*	*C.*
H_2O sol. pepsin	50 c. c.	50 c. c.	50 c. c.
0·2 per cent. HCl....	50	0	0
$C_2H_2O_4$	0	0·5 gram.	1·0 gram.
H_2O.....	0	50 c. c.	50 c. c.
	100	100	100
	0·1 % HCl	0·5 % $C_2H_2O_4$	1·0 % $C_2H_2O_4$

Warmed at 40° C. for 2 hours with 1 gram of pure, dry fibrin, the following results were obtained:

	A.	*B.*	*C.*
Wt. of undigested residue	0·2170	0·4450	0·4371
Per cent. digested.....	78·30	55·45	56·29

A second series, with larger amounts of oxalic acid, gave the following results:

	A.	B.	C.
H_2O sol. pepsin...	50 c. c.	50 c. c.	50 c. c.
0·2 per cent. HCl .	50	0	0
$C_2H_2O_4$	0	1·5 grams.	2·0 grams.
H_2O	0	50 c. c.	50 c. c.
	100	100	100
	0·1 per cent. HCl	1·5 per cent. $C_2H_2O_4$	2·0 per cent. $C_2H_2O_4$
Undigested residue	0·1085 gram.	0·3090 gram.	0·3640 gram.
Per cent. digested	89·15	69·10	63·60

These four results being placed on the same basis, i. e. compared with their respective controls (100) show as follows:

* Jahresbericht für Thierchemie, 1880, p. 309.

† Made in this laboratory by Mr. R. W. Pinney.

Per cent. of acid.	Relative proteolytic action
0·1 HCl	100·0
0·5 $C_2H_2O_4$	70·7
1·0	71·7
1·5	77·5
2·0	71·3

This shows maximum action, with our amount of pepsin, in the presence of 1·5 per cent. of oxalic acid and shows, moreover, that the retarding influence of ammonium oxalate on proteolytic action, is not fully explained by the suggested neutralization of the hydrochloric acid of the gastric juice, unless it be that the ammonium chloride, formed by the reaction, effects pepsin-oxalic acid differently than it does pepsin-hydrochloric acid.

Sodium chloride.

With this salt we obtained, by our method, the following results:

NaCl.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2936 gram.	70·64 per cent.	100·0
0·005 per cent.	0·2824	71·76	101·6
0·010	0·2896	71·04	100·5
0·025	0·3441	65·59	92·8
0·050	0·3511	64·89	91·8
0·100	0·3744	62·56	88·5
0	0·2669	73·31	100·0
0·3	0·4953	50·47	68·8
0·5	0·6175	38·25	52·1
0·8	0·6898	31·02	42·3
1·5	0·7825	21·75	29·6
3·0	0·8249	17·51	23·8

Alex. Schmidt* has recorded the retarding effect of sodium chloride on the proteolytic action of gastric juice; Petit† likewise, has stated that small quantities of sodium chloride interrupt the action of the ferment, and Wolberg‡ has recorded the same result with varying percentages of the salt; noting in addition, that very small quantities cause a slight acceleration of proteolytic action, amounting in his experiment to 2·5 per cent.

With 0·005 per cent. of the salt, we found as the results show, acceleration amounting to 1·6 per cent., larger amounts causing a gradual and proportional diminution in proteolytic action.

* Pflüger's Archiv, xiii, p. 98.

† Jahresbericht für Thierchemie, 1880, p. 309.

‡ Pflüger's Archiv, xxii, p. 298.

Pfeiffer* has also called attention to the retarding action of this substance.

Potassium chloride.

With this salt our results are as follows:

KCl.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1552 gram.	84·48 per cent.	100·0
0·005 per cent.	0·1817	81·83	96·8
0·025	0·1550	84·50	100·0
0·050	0·2565	74·35	88·0
0·100	0·2097	79·03	93·5
0	0·1930	80·70	100·0
0·3	0·3997	60·02	74·3
1·5	0·6815	31·85	39·4
3·0	0·7282	27·18	33·6

The only noticeable difference between the action of this salt and the preceding, is the absence of any acceleration on the part of the potassium chloride and with larger percentages, a less vigorous retarding action.

Ammonium chloride.

For the sake of comparison a few experiments were tried with this salt, with the following results:

$(NH_4)Cl$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·1880 gram.	81·20 per cent.	100·0
0·3 per cent.	0·4649	53·51	65·9
0·8	0·6615	33·85	41·7
3·0	0·6970	30·30	37·3

By looking at the table of comparisons, we see there is little constant difference in the amount of retardation caused by the ammonium, potassium and sodium salts of hydrochloric acid. This result, however, is quite different from that obtained by Wolberg, who found that ammonium chloride influenced proteolytic action but very little, while both potassium and sodium chloride caused great retardation. This difference in result, may be due to difference in strength of gastric juice or to difference in length of time the mixtures were warmed at 40° C.; certainly in our experiments, with pure anhydrous salts and 2 hours digestion, very little difference in digestive action is noticeable; throughout, sodium chloride causes a little less proteolytic action than the corresponding potassium salt, while of the ammonium salt, little is to be said except that it causes equal retardation.

* Centralbl. med. Wiss., 1885, p. 328.

Wolberg in speaking of the relative retarding action of the three sodium salts of hydrochloric, nitric and sulphuric acids, states that the sulphate retards the most, then the nitrate and lastly the chloride. We have noticed the same fact in our work and take it as additional evidence of the liberation of the acid of the salt added, the retarding influence of the salt being dependent, in part, on the digestive power of the pepsin-*acid* formed.

The following experiments,* showing the relative digestive power of the several acids in conjunction with pepsin, testify to the probability of this view.

All of the solutions contained 50 c. c. of an aqueous solution of glycerine-pepsin, each having a total volume of 100 c. c. and differing from each other only in the nature and percentage of the acid present.

In testing the digestive power of the solution, 1 gram of pure fibrin was added and the mixtures warmed at 40° C. for two hours, after which the amount digested was determined in the usual manner.

Percentage of fibrin digested in the presence of the different percentages of acids.

	0·05 %	*0·1 %*	*0·2 %*	*0·3 %*	*0·4 %*	*0·5 %*	Control. *0·1 %* HCl
HNO_3†	9·20	48·75	73·65	67·20	46·00		87·65
H_2SO_4		19·50	24·70	25·80	22·55	24·95	88·05
$C_2H_4O_2$				3·70		2·30	87·50

Comparing these results with their respective controls, figured as 100, we have the following values for sulphuric and nitric acids, expressive of their relative proteolytic power when combined with pepsin, as compared with 0·1 per cent. hydrochloric acid under similar conditions.

Per cent. acid.	HNO_3.	H_2SO_4.
0·05	10·5	----
0·1	55·5	22·1
0·2	84·0	28·0
0·3	76·7	29·1
0·4	52·5	25·6
0·5	----	28·2

From this it is evident, that nitric acid is most active, with our amount of pepsin, in a 0·2 per cent. solution, while sulphuric acid attains its maximum action in 0·3 per cent.; moreover, nitric acid is

* Made in this laboratory by Mr. R. W. Pinney.

† The acids are calculated as pure HNO_3, H_2SO_4, etc.

more than four-fifths as active as 0·1 per cent. hydrochloric acid, while sulphuric acid is only a little more than one-fourth as active as the hydrochloric acid; acetic acid being practically worthless. Hence it is obvious, that, the base being the same, acetates, borates and other salts, the acids of which are not capable of working with pepsin, will most readily retard gastric digestion; then of the other salts experimented with, sulphates and lastly nitrates.

A glance at the table of comparisons, shows here and there, results which manifestly accord with this view, notably lead acetate, cupric sulphate, zinc sulphate,* magnesium sulphate and potassium nitrate.

That this withdrawal of free hydrochloric from the pepsin, is only a partial explanation of the mode of action of neutral salts is plain from the fact that chlorides exert very pronounced retarding action; moreover the action of these latter as well as of the others cannot be due merely to mechanical causes, viz: the semi-saturation of the digestive fluid, since 3·0 per cent. of boracic acid stimulates instead of retarding, and even the presence of 10 per cent. of arsenic acid causes retardation amounting to only 32 per cent. According to Petit,† moreover, saccharose to the extent of 16 per cent. does not interrupt gastric digestion. Again, that the base entering into the composition of the salt has much to do, in many cases, with its retarding proteolytic action, is apparent when we compare the action of ferric chloride, manganous chloride, sodium chloride, mercuric chloride and other metallic salts. That this action is due in part to capability of combining with the proteid matter, thus rendering it non-digestible, is unquestionable.

Acceleration, produced by neutral salts has been noticed by other observers, but no explanation of the cause has been offered. It seems probable, however, that in the case of pepsin-hydrochloric acid, one plausible reason, at least, may be suggested. If, as has been mentioned, many of the neutral salts are decomposed by the acid of the gastric juice, it would follow that the addition of very small amounts of the salts would diminish slightly the percentage of free hydrochloric acid, while the acid liberated from the salt, would be entirely without deleterious action. Nearly every experimenter in this direction has employed in the preparation of gastric juice 0·2 per cent. hydrochloric acid, which is well adapted for the purpose, but the action of the acid is dependent in part on the amount of ferment. With our solution of pepsin, the following results, expressed in the percentage of fibrin

* Compare Petit, Jahresbericht für Thierchemie, 1880, p. 309.

† Jahresbericht für Thierchemie, 1880, p. 309.

digested, were obtained with different percentages of hydrochloric acid; the amount of pepsin being the same in each case.

Per cent. HCl.....	*0·05*	*0·1*	*0·2*	*0·3*	*0·4*
Per cent. fibrin digested	73·8	*89·3*	84·0	81·7	63·8

This shows plainly, that, with the amount of pepsin employed in our experiments, acceleration of proteolytic action on the addition of neutral salts, might in some instances be due to slight diminution of free hydrochloric acid. That this is not the sole cause, however, is plain from the fact that closely related salts do not act alike.

Potassium bromide and Potassium iodide.

With these two potassium salts, the following results were obtained:

KBr.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3837 gram.	61·63 per cent.	100·0
0·005 per cent.	0·3205	67·95	110·2
0·025	0·3035	69·65	113·0
0·10	0·4204	57·96	94·0
0·50	0·5690	43·10	70·0
1·00	0·6203	37·97	61·6
KI.			
0	0·2572 gram.	74·28 per cent.	100·0
0·005 per cent.	0·2755	72·45	97·5
0·025	0·3421	65·78	88·6
0·10	0·2841	71·59	96·4
0·50	0·6367	36·33	48·9
1·00	0·7586	24·14	32·5

By comparison of these two series of results, we see that there is a very decided difference in the action of the two salts; potassium bromide in very small quantity, causes a decided acceleration in proteolytic action, which is wholly lacking with potassium iodide. Again, there is a very great difference in the amount of retardation produced by the two salts; thus by comparison with their respective controls we see, that while 1 per cent. of potassium bromide causes retardation in digestive action, amounting to 38·4 per cent., the same percentage of iodide causes retardation amounting to 67·5 per cent. It is to be supposed, naturally, that in the presence of large amounts of the salts, the ferment must act wholly in combination with hydrobromic and hydriodic acid respectively; that is to say, the hydrochloric acid of the gastric juice must have replaced these two acids in the potassium salts, and thus new pepsin-compounds formed, of which pepsin-hydrobromic acid is the more active digestive agent.

Putzeys,* indeed, found that he obtained practically the same proteolytic action [retarding] with pepsin and hydriodic acid, as with potassium iodide and pepsin-hydrochloric acid. The following results, showing the relative digestive action of pepsin with hydrobromic and hydriodic acid respectively, were obtained by Putzeys.

HI.	HBr.	Digestive products formed.
0·625 gram.	----	15·86 per cent.
0·937	----	31·33
3·310	----	2·33
----	0·882 gram.	26·40
----	2·200	45·60
----	3·309	46·60

It is thus evident, that both hydrobromic and hydriodic acid can, to a certain extent, replace the hydrochloric acid of the gastric juice, although they are much less active than the latter acid. Moreover, hydrobromic acid is much more efficient than hydriodic in connection with the ferment, for in comparatively large doses the latter acid will completely stop proteolytic action. As a practical result, Putzeys suggests, that for therapeutic purposes, potassium bromide and iodide should be given ½ to 1 hour before meals. Our results are plainly in accord with Putzeys' observations.

The following table shows the relative influence on the proteolytic action of pepsin, of the various inorganic salts experimented with, compared with the controls, expressed as 100.

* Jahresbericht für Thierchemie, 1877, 279. De l'influence de l'iodure et du bromure de potassium sur la digestion stomacale.

Table showing relative proteolytic action.

	0·001 p.c.	0·005 p.c.	0·01 p.c.	0·025 p.c.	0·05 p.c.	0·1 p.c.	0·2 p.c.	0·3 p.c.	0·5 p.c.	0·8 p.c.	1·0 p.c.	1·5 p.c.	2·0 p.c.	3·0 p.c.	5·0 p.c.	10·0 p.c.
$HgCl_2$	93·8	92·7	----	----	----	60·2	----	----	11·4	----	0	----	----	----	----	----
$HgBr_2$	----	97·8	----	93·9	----	----	----	----	----	----	----	----	----	----	----	----
HgI_2	----	107·4	----	95·1	----	----	----	----	----	----	----	----	----	----	----	----
$Hg(CN)_2$	----	107·5	----	93·8	----	106·3	----	----	----	----	----	----	----	----	----	----
$CuSO_4 + 5H_2O$	104·8	102·8	97·0	86·1	85·6	61·2	----	31·5	26·4	23·3	----	19·8	----	----	----	----
$Pb(C_2H_3O_2)_2 + 3H_2O$	104·2	100·5	101·9	103·0	----	97·8	----	69·6	32·8	2·8	----	0·7	----	0	----	----
$SnCl_2$	----	----	----	97·5	----	69·6	----	----	35·9	----	24·8	----	13·3	----	----	----
As_2O_3	----	----	----	----	103·0	99·3	102·6	----	105·1	----	----	----	----	----	----	----
H_3AsO_4	----	----	----	----	----	----	101·2	----	101·6	----	102·0	----	98·2	----	90·8	68·1
$ZnSO_4 + 7H_2O$	101·6	101·5	96·2	89·9	----	61·9	----	34·4	31·4	27·9	27·2	21·6	----	19·3	----	----
Fe_2Cl_6	96·7	97·3	96·0	----	93·9	----	----	43·2	24·4	15·0	----	----	----	5·2	----	----
$FeSO_4 + 7H_2O$	99·0	95·0	99·2	90·9	88·5	81·0	----	33·8	----	23·8	----	19·2	----	----	----	----
$MnCl_2$	98·7	----	101·3	98·2	100·8	----	----	77·7	----	43·8	----	41·7	----	32·0	----	----
$MgSO_4 + 7H_2O$	----	90·7	86·0	----	75·5	62·3	----	33·1	28·5	23·7	----	15·0	----	14·9	----	----
$K_2Mn_2O_8$	----	21·3	0·6	----	----	----	----	----	----	----	----	----	----	----	----	----
$K_2Cr_2O_7$	----	----	94·4	----	----	45·3	----	----	----	----	----	----	----	----	----	----
KCN	----	----	----	90·5	----	----	----	----	1·3	----	----	----	----	----	----	----
$K_4Fe(CN)_6 + 3H_2O$	----	93·3	----	94·5	64·1	33·0	----	----	0	----	----	----	----	----	----	----
$(NH_4)_2C_2O_4 + 2H_2O$	----	----	95·1	94·6	----	90·1	----	----	14·1	----	0·6	----	----	----	----	----
$Na_2B_4O_7 + 10H_2O$	----	----	----	----	96·2	----	92·6	----	35·8	----	1·5	----	----	104·2	----	----
H_3BO_3	----	----	----	----	----	102·1	----	----	102·2	----	----	----	----	----	----	----
$KClO_3$	----	----	----	----	----	----	----	74·3	----	40·7	----	25·0	----	17·6	----	----
KNO_3	----	----	----	----	----	----	----	64·9	52·6	39·8	----	25·9	----	15·3	----	----
KCl	----	96·8	----	100·0	88·0	93·5	----	74·3	----	----	----	39·4	----	33·6	----	----
NaCl	----	101·6	100·5	92·8	91·8	88·5	----	68·8	52·1	42·3	----	29·6	----	23·8	----	----
$(NH_4)Cl$	----	----	----	----	----	----	----	65·9	----	41·7	----	----	----	37·3	----	----
KBr	----	110·2	----	113·0	----	94·0	----	----	70·0	----	61·6	----	----	----	----	----
KI	----	97·5	----	88·6	----	96·4	----	----	48·9	----	32·5	----	----	----	----	----

Alkaloid salts.

According to Petit, alkaloids do not interrupt the action of pepsin in acid solution. Wolberg, however, found that morphine chloride, strychnine, quinine sulphate and narcotine, used in very small quantities (0·5 grain to 100 c. c.), caused in every instance, excepting with quinine sulphate, retardation amounting on an average to nearly 3

per cent.; with quinine sulphate, slight acceleration was obtained. Strychnine and narcotine, in very small quantity, gave slight acceleration. Dr. H. v. Boeck* states, that quinine is without influence on the activity of pepsin.

In our experiments, using larger amounts of the alkaloid salts, retardation is very pronounced, doubtless due in part to replacement of the sulphuric acid of the salts by the acid of the gastric juice.

Alkaloid salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3555 gram.	64·45 per cent.	100·0
$(Sr)_2 . H_2SO_4 + 6H_2O$.			
0·5 per cent.	0·6825	31·75	49·2
1·0	0·8213	17·87	27·7
$(Br)_2 . H_2SO_4 + H_2O$.			
0·5	0·5685	43·15	66·9
1·0	0·7700	23·00	35·6
$(At)_2 . H_2SO_4$.			
0·5	0·6412	35·88	55·6
$(Q)_2 . H_2SO_4 + 7H_2O$.			
0·5	0·6606	33·94	52·6
$(Ci)_2 + H_2SO_4 + 2H_2O$.			
0·5	0·6885	31·15	48·3
$(Mo)_2 . H_2SO_4 + 5H_2O$.			
0·5	0·6225	37·75	58·5
$(Na)_2 . H_2SO_4 + H_2O$.			
0·5	0·6365	36·35	56·4

Percentage retardation of the alkaloid salts (0·5 *per cent.*)

Strychnine	50·8
Brucine	33·1
Atropine	44·4
Quinine	47·4
Cinchonine	51·7
Morphine	41·5
Narcotine	43·6

* Zeitschrift für Biologie, vol. vii, p. 428.

Influence of Various Therapeutic and Toxic Substances on the Proteolytic Action of the Pancreatic Ferment.

By R. H. Chittenden and Geo. W. Cummins, Ph.B.

With the proteolytic ferment of the pancreatic juice, few systematic experiments have been tried to ascertain the influence of those substances the frequency of whose use, as therapeutic or toxic agents, renders their action on this ferment a matter of no small consequence. The well known experiments of Heidenhain, Kühne and Schmidt have been confined to the action of sodium carbonate, sodium chloride and kindred salts of physiological importance. Pfeiffer* has indeed, in addition, experimented with a few of the sulphates of the alkalies and alkali-earths, but of the large number of metallic and other salts in common use, few have been tried, while the action of alkaloids and the gases occurring in the intestinal canal, has not been studied at all.

Method employed.

It is a characteristic of the proteolytic ferment of the pancreatic juice, that it exercises considerable digestive power in a *neutral* solution.† In view therefore of the fact that the ferment, while in the intestinal canal, may be obliged to act in an acid-reacting medium (due to acid-proteids) as frequently as in an alkaline or neutral one, it seemed advisable in the experiments, to employ a neutral solution of trypsin. Moreover, it becomes necessary to use such a solution, if salts of the heavy metals are to be experimented with, since in alkaline solutions, carbonates or oxides of the metals would be formed and thus affect the results; still, again, the action of all substances can be best studied in neutral solution, since under such conditions, no replacements or decompositions of any kind take place to complicate the action of the substance, other than the direct action on the ferment or the proteid matter. Hence, a neutral solution has in every case been employed as being the most satisfactory, while only such substances have been experimented with, as contained no free acid or alkali, the destructive action of which is well known.

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* Centralbl. med. Wiss., 1885, p. 328.

† See Chittenden and Cummins, Amer. Chem. Jour., vol. vii, p. 46. Also Trans. Conn. Acad., vol. vii.

The solution of trypsin was prepared according to Kühne's* method from dried ox pancreas freed from fat; 40 grams dry pancreas in 500 c. c. 0·1 per cent. salicylic acid, neutralized and diluted to 2 litres.† In each digestion experiment, 25 c. c. of this neutral trypsin solution were used, to which was added 25 c. c. of water containing the substance to be experimented with, or in the control 25 c. c. of water alone, making the volume of the digestive mixture in each case 50 c. c. In testing the proteolytic action of the different solutions, 1 gram of pure dry, pulverized fibrin, described in the preceding article, was added and the mixtures warmed at 40° C. for six hours, after which the undigested fibrin was filtered on weighed filters, washed thoroughly and dried at 100–110° C. until of constant weight.

In this work as in the study of the salivary ptyalin and pepsin, it has been the effort mainly to ascertain the relative action of small quantities of the various salts, rather than the percentages necessary to completely stop the action of the ferment, this to our mind being much the more important.

Mercuric chloride.

With small percentages of this salt, the following results were obtained:

$HgCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4495 gram.	55·05 per cent.	100·0
0·002 per cent.	0·4465	55·35	100·5
0·003	0·4405	55·95	101·6
0·005	0·4562	54·38	98·7
0·025	0·5076	49·24	89·4
0·100	0·7753	22·47	40·8

As seen from the table, 0·1 per cent. mercuric chloride diminishes the proteolytic action of the ferment more than one-half. Its action in this percentage is more energetic than on pepsin, although the smaller quantities are proportionally far less active; in one case (0·003 per cent.) even causing acceleration.

Wassilieff‡ has studied the influence of mercurous chloride on pancreatic digestion and finds that calomel does not affect the action of the proteolytic ferment, while it does prevent the formation of putrefaction products, viz: indol, phenol, etc.

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* Untersuchungen aus der physiolog. Inst. d. Universität Heidelberg, vol. i, p. 222.

† The solution was kept from putrefaction by the use of a little 20 per cent. alcoholic solution of thymol; enough of which remained dissolved in the fluid to prevent putrefaction also during the six hours digestion at 40° C.

‡ Zeitschrift für physiol. Chemie, vol. vi, p. 112.

Mercuric iodide and Mercuric bromide.

These salts dissolved in sodium chloride in such proportion that the ultimate solutions contained like percentages of both salts, gave the following results:

HgI_2.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4707 gram.	52·93 per cent.	100·0
0·005 per cent.	0·4780	52·20	98·6
0·025	0·5085	49·15	92·8
0·100	0·5994	40·06	75·6
0·200	0·6580	34·20	64·6
$HgBr_2$.			
0	0·4707 gram.	52·93 per cent.	100·0
0·005 per cent	0·4400	56·00	105·8
0·025	0·4840	51·60	97·4
0·100	0·5721	42·79	80·8
0·200	0·6548	34·52	65·2

Aside from a slight acceleration, noticed with 0·005 per cent. of bromide, the two salts act very much alike, causing retardation of proteolytic action; less pronounced however, than that caused by mercuric chloride.

Mercuric cyanide.

The action of this salt is somewhat peculiar, causing at first when in small quantity, noticeable retardation followed in the presence of larger percentages by increased proteolytic action, though still below the action of the normal solution of trypsin.

In a general way, its action on this ferment accords very nearly with its action on the amylolytic ferment of saliva and the proteolytic ferment of the gastric juice.

$Hg(CN)_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2668 gram.	73·32 per cent.	100·0
0·005 per cent.	0·3192	68·08	92·8
0·025	0·3209	67·91	92·6
0·050	0·3244	67·56	92·1
0·100	0·3308	66·92	91·2
0	0·3675 gram.	63·25 per cent.	100·0
0·3 per cent.	0·4094	59·06	93·3
0·5	0·3880	61·20	96·7
1·5	0·3932	60·68	95·9

Its action is to be attributed mainly to the hydrocyanic acid radical, judging from the action of potassium cyanide on the one hand and the action of mercury salts on the other. There is, however, without doubt a close connection between the action of these salts and their power of combining with proteid matter in general.

Cupric sulphate.

With this salt a more energetic retarding action is to be noticed, than in the case of the proteolytic ferment of gastric juice; 0·1 per cent. of the salt causing a retardation in proteolytic action of 65·7 per cent. as compared with a retardation of 38·8 per cent. in the case of pepsin.

$CuSO_4+5H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·5035 gram.	49·65 per cent.	100·0
0·005 per cent.	0·5027	49·73	100·1
0·025	0·5320	46·80	94·2
0·050	0·5856	41·44	83·4
0·100	0·8295	17·05	34·3

Lead acetate.

With this salt the following results were obtained, agreeing essentially in the smaller percentages, with those obtained with cupric sulphate. In the presence of 0·1 per cent., however, retardation is far less than with the same percentage of cupric sulphate; 0·5 per cent. completely stops proteolytic action.

$Pb(C_2H_3O_2)_2+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4562 gram.	54·38 per cent.	100·0
0·005 per cent.	0·4655	53·45	98·2
0·025	0·5391	46·09	84·7
0·050	0·5660	43·40	79·8
0·100	0·6410	35 90	66·0
0·500	1·0	0	0

Stannous chloride.

This salt, in conformity with its action on the amylolytic ferment of saliva, causes very decided retardation in the proteolytic action of trypsin, requiring but a very small amount of the salt to completely stop the action of the ferment.

$SnCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3875 gram.	61·25 per cent.	100·0
0·0005 per cent.	0·4495	55·05	89·8
0·005	0·5370	46·30	75·5
0·025	1·0	0	0

Arsenious oxide.

Schäfer and Böhm have experimented with arsenious acid, studying its influence on the proteolytic action of a glycerine infusion of

* Abstract in Jahresbericht für Thierchemie, 1872, p. 363.

pancreas. They find that arsenious acid is without influence on the action of the ferment. We do not know whether they added arsenious acid to a neutral or alkaline solution of the ferment, as we have seen only an abstract of their paper, but we find that the addition of very small amounts of arsenious oxide to a neutral solution of trypsin, does produce a slight retardation in the proteolytic action of the ferment. Owing to the comparative insolubility of arsenious oxide in water, only small percentages could be employed, but these show, as might be expected from the acid character of the substance, a decrease in proteolytic action.

As_2O_3.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4598 gram.	54·02 per cent.	100·0
0·001 per cent.	0·4630	53·70	99·4
0·005	0·4513	54·87	101·5
0·025	0·4710	52·90	97·9
0·050	0·4723	52·77	97·6

Arsenic acid.

This substance, in conformity with its more decided acid character, causes greater retardation than arsenious acid; which fact tends, by analogy, to make the retarding action of the latter still more probable. The results are as follows:

H_3AsO_4.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4598 gram.	54·02 per cent.	100·0
0·005 per cent.	0·4563	54·37	100·6
0·025	0·4887	51·13	94·6
0·050	0·5264	47·36	87·4
0·100	0·6340	36·60	67·7

Ammonium arsenate.

This salt in small amount, appears to increase the proteolytic action of the ferment, which fact would indicate that the retarding action of the two preceding arsenic compounds is due more to their acid character than to the arsenic contained in them.

$(NH_4)_2AsO_4$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4598 gram.	54·02 per cent.	100·0
0·050 per cent.	0·4620	53·80	99·6
0·100	0·4442	55·58	102·8
0	0·3675	63·25	100·0
0·5	0·4071	59·29	93·7

Potassium antimony tartrate.

This compound of antimony fails to produce with trypsin the marked acceleration, noticed with the amylolytic ferment studied.

This difference in action, since both ferments were in neutral solution, indicates a specific difference in the nature of the two ferments. Following are the results obtained:

$K(SbO)C_4H_4O_6$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3978 gram.	60·22 per cent.	100·0
0·2 per cent.	0·4105	58·95	97·8
0·5	0·4129	58·71	97·4
1·0	0·4258	57·42	95·3
1·5	0·5406	45·94	76·2

Ferric chloride and ferrous sulphate.

With these two salts of iron the following results were obtained:

Fe_2Cl_6.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·5215 gram.	47·85 per cent.	100·0
0·005 per cent.	0·5431	45·69	95·4
0·025	0·6243	37·57	78·5
0·050	0·7457	25·43	53·1
0·100	1·0	0	0
$FeSO_4+7H_2O$.			
0	0·4075 gram.	59·25 per cent.	100·0
0·005 per cent.	0·4548	54·52	92·0
0·05	0·6225	37·75	63·7
0·10	0·7177	28·23	47·6
0·25	0·7104	28·96	48·8
0·50	0·7219	27·81	46·9
1·00	0·7675	23·25	39·2
1·50	0·7861	21·39	36·1

Ferric chloride is seen to be far more energetic in its hindering action on the proteolytic ferment, than the ferrous salt. A like result was obtained with the amylolytic ferment of the saliva and to a lesser extent with pepsin-hydrochloric acid.

Bubnow* has tried experiments with both of these salts and found, as might be expected, that when present to the extent of 5 per cent. they prevented the appearance of putrefaction products (indol, phenol, etc.) but did not interfere with the action of the unorganized ferment. As no quantitative results were obtained, we can make no direct comparisons. In our experiments, it is to be remembered that putrefaction is prevented by thymol.

* Zeitschrift für physiol. Chemie., vol. vii, p. 327.

Manganous chloride.

Following are the results obtained with this salt:

$MnCl_2$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·5215 gram.	47·85 per cent.	100·0
0·005 per cent.	0·5201	47·99	100·3
0·050	0·5139	48·61	101·6
0·100	0·5483	45·17	94·4
0·500	0·6807	31·93	66·7

Comparing the influence of this salt on the proteolytic action of trypsin, with the results obtained with the closely related iron salts, it is seen that the manganese salt has a far less retarding influence than the latter; a fact which agrees with what was observed in studying its action on pepsin-hydrochloric acid.

Consequently, manganese salts, in so far as they are adapted to replace iron salts as therapeutic agents, are apparently less liable to interfere with normal digestive action.

Zinc sulphate.

$ZnSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·5035 gram.	49·65 per cent.	100·0
0·005 per cent.	0·5175	48·25	97·2
0·025	0·5805	41·95	84·5
0·050	0·6802	31·98	64·4
0·100	0·8301	16·99	34·2

The action of this salt on trypsin is, both in character and extent, similar to its action on the amylolytic ferment of saliva. Compared with pepsin-hydrochloric acid, however, the action of the salt is seen to be more energetic on the pancreatic ferment.

Barium chloride.

The following results were obtained:

$BaCl_2+2H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3710 gram.	62·90 per cent.	100·0
0·05 per cent.	0·3515	64·85	103·1
0·5	0·3885	61·15	97·2
3·0	0·4986	50·14	79·7

With this salt a slight acceleration in proteolytic action is to be seen with 0·05 per cent., while the larger amounts cause noticeable retardation.

Magnesium sulphate.

$MgSO_4+7H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3495 gram.	65·05 per cent.	100·0
0·05 per cent.	0·3541	64·59	99·3
0·5	0·3810	61·90	95·1
3·0	0·4706	52·94	81·3

Here retarding action is similar in extent to the action of barium chloride. Compared with zinc sulphate, the difference in action on trypsin is about the same in extent as the difference found in the action of the two salts on the amylolytic ferment of saliva. The retarding action of this salt on pancreatic digestion has been previously noticed by Pfeiffer.*

Potassium permanganate.

The retarding action of potassium permanganate is very pronounced. There is, however, a noticeable difference between the action of the salt on trypsin and its action on pepsin and ptyalin, the two latter being much more readily affected by the permanganate; that is, by much smaller percentages.

$K_2Mn^2O_8$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4562 gram.	54·38 per cent.	100·0
0·001 per cent.	0·4570	54·30	99·8
0·003	0·4608	53·92	99·1
0·010	0·4833	51·67	95·0
0	0·3675	63·25	100·0
0·1	0·4487	55·13	87·1
0·5	0·7769	22·31	35·2
1·0	1·0	0	0

Potassium dichromate.

$K_2Cr_2O_7$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3978 gram.	60·22 per cent.	100·0
0·05 per cent.	0·4032	59·68	99·1
0·2	0·4245	57·55	95·5
0·5	0·4693	53·07	88·1
1·0	0·5525	44·75	74·3
1·5	0·6321	36·79	61·0

The retarding action of this salt is proportionally greater than that of the other potassium salts, indicating that the acid has a specific action of its own. Moreover, being an acid salt, the acid radical is present in larger amount than in the neutral potassium salts experimented with.

Potassium cyanide.

The very pronounced acceleration in proteolytic action caused by the larger percentages of this salt is very interesting, especially when we recall the fact, that the retarding action of the same salt on the

* Centralbl. med. Wiss., 1885, p. 328.

amylolytic ferment of saliva and on the proteolytic ferment of gastric juice was equally decided, even with very small pecentages.

KCN.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2668 gram.	73·32 per cent.	100·0
0·005 per cent.	0·2790	72·10	98·3
0·025	0·2840	71·60	97·6
0·050	0·2678	73·22	99·8
0·100	0·2600	74·00	100·9
0	0·3675	63·25	100·0
0·3	0·2076	79·24	125·2
0·5	0·1985	80·15	126·7
1·0	0·2697*	73·03	115·4
1·5	0·3315*	66·85	105·6

Potassium ferrocyanide and Potassium ferricyanide.

With these two salts the following results were obtained:

$K_4Fe(CN)_6+3H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3045 gram.	69·55 per cent.	100·0
0·005 per cent.	0·3363	66·37	95·4
0·50	0·3293	67·07	96·4
2·00	0·3753	62·47	89·8
$K_6Fe_2(CN)_{12}$			
0·005 per cent.	0·3268	67·32	96·8
0·05	0·3370	66·30	95·3
2·00	0·3912	60·88	87·5

Here, unlike the cyanide, there is no acceleration in the proteolytic action of the ferment, neither on the other hand is there very pronounced retardation; less indeed than is produced by a like percentage (2·0) of sodium chloride.

Sodium tetraborate.

With this salt we obtained the following results:

$Na_2B_4O_7+10H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·4116 gram.	58·84 per cent.	100·0
0·05 per cent.	0·3729	62·71	106·5
0·2	0·3260	67·40	114·5
0·5	0·2332	76·68	130·3
1·0	0·1860	81·40	138·3
2·0	0·1663	83·37	141.7
3·0	0·2276	77·24	131·2
5·0	0·3141	68·59	116·5

* It was impossible to wash these completely, consequently the weights are undoubtedly too high.

Here, unlike the action of the salt on the two preceding ferments studied, there is to be seen a gradual increase in proteolytic action, which is very pronounced in the presence of 2 per cent. of the salt, then gradually diminishes; although even with 5 per cent. of the crystallized salt, the action of the ferment continues to be increased far above that of the normal juice.

Sodium sulphate.

Following are the results obtained:

$Na_2SO_4+10H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3495 gram.	65·05 per cent.	100·0
0·05 per cent.	0·3602	63·98	98·3
0·5	0·3558	64·42	99·0
2·0	0·3938	60·62	93·1
5·0	0·4360	56·40	86·7

The retarding action of this salt is quite pronounced, although it is noticeable that its retarding action is not equal to that of magnesium sulphate nor indeed to that of sodium chloride. Pfeiffer* has also noticed the retarding action of this salt. Weiss,† however, has stated that sodium sulphate in very small quantity increases the proteolytic activity of a pancreas extract. As we have not seen the original article we do not know whether definite percentages were employed or not; with 0·05 per cent. of the crystallized salt, under the conditions of our experiment, there was certainly no acceleration in proteolytic action.

Potassium chlorate and Potassium nitrate.

	Undigested residue.	Fibrin digested.	Relative proteolytic action.
$KClO_3$.			
0	0·3662 gram.	63·38 per cent.	100·0
0·05 per cent.	0·3991	60·09	94·8
0·50	0·3743	62·57	98·7
2·00	0·4101	58·99	93·0
KNO_3.			
0	0·3710 gram.	62·90 per cent.	100·0
0·05 per cent.	0·3751	62·49	99·3
0·5	0·3804	61·96	98·5
2·0	0·4080	59·20	94·1
5·0	0·4473	55·27	87·8

* Loc. cit.

† Jahresbericht für Thierchemie, 1876, p. 177.

The effects of these two salts on the proteolytic action of the ferment are very similar, with the exception that potassium chlorate appears to be a little more energetic in action than the nitrate. Weiss* has stated that a very small quantity of potassium nitrate added to a pancreas infusion, causes slight increase in the proteolytic activity of the ferment. This, however, is not observable in our experiment with 0·05 per cent., although retardation is very slight.

Potassium chloride.

This salt, unlike sodium chloride, appears to cause retardation of proteolytic action, and is without any accelerating influence whatever. The extent of its retardation, however, is not so great as in the case of sodium chloride. Following are the results obtained.

KCl.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3662 gram.	63·38 per cent.	100·0
0·05 per cent.	0·3740	62·60	98·7
0·5	0·3672	63·27	99·8
2·0	0·4331	56·69	89·4
5·0	0·4723	52·77	83·2

Sodium chloride.

With this salt many previous experiments have been tried; Heidenhain,† after noting the accelerating action of sodium carbonate, tried the influence of sodium chloride and found that it increased the proteolytic power of the ferment. E. Pfeiffer, however, states that he found sodium chloride to exert a retarding influence on the proteolytic action of the pancreatic ferment. Pfeiffer‡ moreover states that he found sodium carbonate to exert its accelerating action only when present in 0·5 per cent., not in 0·24 per cent. as found by Heidenhain. Recent results§ obtained by us in another connection, however, fully substantiate Heidenhain's statements on this point. Lastly, Lindberger‖ has found that sodium chloride, which in a neutral or alkaline solution of trypsin accelerates its proteolytic action, causes in an acid solution (0·018 per cent. HCl, presumably as acid-proteid) marked retardation of ferment action, without, however,

* Loc. cit.

† Beiträge zur Kenntniss des Pancreas, Pflüger's Archiv, vol. x, p. 578.

‡ Loc. cit.

§ Chittenden and Cummins, Amer. Chem. Jour., vol. vii, p. 46–47. Also Trans. Conn. Acad., vol. vii.

‖ Jahresbericht für Thierchemie, 1883, p. 281.

causing destruction of the ferment, as after dialysis the ferment was again active.

Our results accord in the main with Heidenhain's; acceleration, followed by retardation of proteolytic action as seen from the following figures.

NaCl.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·2993 gram.	70·07 per cent.	100·0
0·05 per cent.	0·2635	73·65	105·1
0·2	0·3001	69·99	99·8
0·5	0·3372	66·28	94·5
1·0	0·3923	60·77	86·7
2·0	0·4352	56·48	80·6
3·0	0·4361	56·39	80·4
5·0	0·4740	52·60	75·0

Potassium bromide and potassium iodide.

The action of these two salts is wholly an accelerating one; more pronounced, however, in the case of the bromide than in the iodide.

Following are the results of the experiments:

KBr.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3881 gram.	61·19 per cent.	100·0
0·05 per cent.	0·3773	62·20	101·6
0·5	0·3681	63·19	103·2
2·0	0·3795	62·05	101·4
5·0	0·3278	67·22	109·8
KI.			
0	0·3881 gram.	61·19 per cent.	100·0
0·05 per cent.	0·3575	64·25	105·0
0·5	0·3915	60·85	99·4
3·0	0·3897	61·03	99·7

Influence of gases on the proteolytic action of trypsin.

Podolinski* in Heidenhain's laboratory, has shown that oxygen gas has the power of converting the zymogen of pancreas into the active ferment and that carbonic acid is without such power; moreover, that carbonic acid added to a sodium carbonate solution of pancreatin (trypsin) retards the proteolytic action of the ferment, because the normal carbonate is thus changed into acid carbonate which does not favor the action of the ferment so well as the simple salt. Hydrogen was found to be without such action. Podolinski further found that while oxygen favored the conversion of zymogen into the ferment, it did not influence the proteolytic action

* Pflüger's Archiv, vol. xiii, p. 426.

the ferment. Hydrogen was also found to be without marked action on the ferment, but carbonic acid retarded decidedly the proteolytic action of the ferment in an alkaline solution.

We have tried the influence of three gases, such as the ferment would naturally be brought in contact with in the intestinal canal, and find that they exert a marked influence on the activity of the ferment. We employed, as in the preceding experiments, a neutral solution of trypsin, and kept the solutions saturated with the gas during the experiment, by passing a constant current through the fluid contained in a small flask. As a control, air was passed through one digestive mixture, which served to keep the powdered fibrin in an equal state of agitation and thus make accurate comparison possible.

Following are the results:

	Undigested residue.	Fibrin digested.	Relative proteolytic action.
Air	0·4218 gram.	57·82 per cent.	100·0
Hydrogen (H)	0·3670	63·30	109·1
Carbonic acid (CO_2)	0·5665	43·35	74·9
Hydrogen sulphide (H_2S)	0·4884	51·16	88·5

From this it is seen that hydrogen gas causes a slight acceleration in the proteolytic action of the ferment, while carbonic acid causes marked retardation, which fact agrees with Podolinski's results, and shows moreover, that the action of the gas is the same in neutral and alkaline solutions. Hydrogen sulphide also causes retardation, although not so marked as carbonic acid.

Influence of alkaloid salts on the proteolytic action of trypsin.

So far as we know, no previous experiments have been tried on this subject. Our results show, so far as our experiments extend, a greater susceptibility on the part of trypsin to the action of alkaloids than the amylolytic ferment of saliva. Moreover, with trypsin, the alkaloids in only one case cause acceleration of proteolytic action. Compared with pepsin-hydrochloric acid, however, we find that neutral solutions of trypsin are not so readily affected as the former ferment, except by one alkaloid, viz: narcotine, where retarding action is very pronounced. All of the alkaloids experimented with were in the form of pure sulphates.

Morphine sulphate.

This salt exerts but little retarding action. The results are as follows:

$(Mo)_2 . H_2SO_4 + 5H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3875 gram.	61·25 per cent.	100·0
0·05 per cent.	0·3952	60·48	98.7
0·5	0·4105	58·95	96·2
2·0	0·4542	54·58	89·1

Atropine sulphate.

This alkaloid is very similar to morphine in its action.

$(At)_2 . H_2SO_4$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·3875 gram.	61·25 per cent.	100·0
0·05 per cent.	0·3909	60·91	99·4
0·5	0·4078	59·22	96·6
2·0	0·4619	53·81	87·8

Stolnikow* has stated that a very small amount of atropine sulphate is without influence on the ferment power of a glycerine extract of pancreas, but that large amounts of the alkaloid salt, considerably diminish the proteolytic action of the ferment.

Strychnine sulphate and Brucine sulphate.

These two alkaloids produce more marked effects on the ferment, than the preceding. Of the two, as the results show, strychnine is more powerful in preventing proteolytic action.

Alkaloid salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4511 gram.	54·89 per cent.	100·0
$(Sr)_2 . H_2SO_4 + 6H_2O$.			
0·05 per cent.	0·4760	52·40	95·4
0·5	0·5792	42·08	76·6
2·0	0·6882	31·18	56·8
$(Br)_2 . H_2SO_4 + H_2O$.			
0·05	0·4915	50·85	92·6
0·5	0·5480	45·20	82·3
2·0	0·5452	45·48	82·8

Narcotine sulphate.

$(Na)_2 . H_2SO_4 + H_2O$.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4511 gram.	54·89 per cent.	100·0
0·05 per cent.	0·5205	47·95	87·3
0·5	0·9943	0·57	1·0
2·0	1·0	0	0

This alkaloid causes a complete stopping of proteolytic action; nearly so, even when present to the extent of only 0·5 per cent.

* Virchow's Archiv, vol. xc, p. 435.

Quinine sulphate, Cinchonine sulphate and Cinchonidine sulphate.

The following table of results shows the action of these three salts:

Alkaloid salt.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·4679 gram.	53·21 per cent.	100·0
$(Q)_2 . H_2SO_4 + 7H_2O$.			
0·05 per cent.	0·4811	51·89	97·5
0·5	0·6310	36·90	69·3
2·0	0·7600	24·00	45·1
$(Ci)_2 . H_2SO_4 + 2H_2O$.			
0·05	0·4868	51·32	96·4
0·5	0·6409	35·91	67·4
2·0	0·8327	16·73	31·4
$(Cidine)_2 . H_2SO_4 + 3H_2O$.			
0·05	0·4467	55·33	104·0
0·5	0·5977	40·23	75·6
2·0	0·7707	22·93	43·0

Cinchonidine, in the smallest percentage, causes a slight acceleration in proteolytic action; retardation is also less marked with 0·5 per cent. than in the case of the other two alkaloids. Otherwise the results are much alike.

On the opposite page is a table showing relative action of the various salts on the proteolytic power of the ferment, compared with the action of the controls expressed as 100.

O. Nasse,* by a study of the influence of various salts (inorganic) on fermentation, particularly their influence on the amylolytic action of the salivary ferment, pancreatic ferment, and the invert ferment of yeast, came to the conclusion that there is a manifest and important dependence on the part of the ferments in question, in their action, on the presence of salt molecules, and moreover, that this dependence is specific for each individual ferment; that the quantity, as well as the quality of a salt, exercises a specific influence upon each ferment.

Our own results, with still different ferments, and with a larger number of substances, both related and more varied in character, all testify to the truth of this statement, viz: that the unorganized ferments are much influenced by the presence of salts, and moreover, that there is no distinct relationship among the ferments in question in their behavior towards the various salts experimented with, as a study of the three tables of comparisons show. Thus one and the same salt may affect two ferments in quite a different manner, as seen

* Pflüger's Archiv, vol. xi, p. 157.

Table showing relative proteolytic action.

	0·0005 p. c.	0·001 p. c.	0·002 p. c.	0·003 p. c.	0·005 p. c.	0·010 p. c.	0·025 p. c.	0·05 p. c.	0·1 p. c.	0·2 p. c.	0·3 p. c.	0·5 p. c.	1·0 p. c.	1·5 p. c.	2·0 p. c.	3·0 p. c.	5·0 p. c.
$HgCl_2$			100·5	101·6	98·7		89·4		40·8								
$HgBr_2$					105·8		97·4		80·8	65·2							
HgI_2					98·6		92·8		75·6	64·6							
$Hg(CN)_2$					92·8		92·6	92·1	91·2		93·3	93·7		95·9			
$CuSO_4 + 5H_2O$					100·1		94·2	83·4	34·3								
$Pb(C_2H_3O_3)_2 + 3H_2O$					98·2		84·7	79·8	66·0			0					
$SnCl_2$	89·8				75·5		0										
As_2O_3		99·4			101·5		97·9	97·6									
H_3AsO_4					100·6		94·6	87·4	67·7								
$(NH_4)_3AsO_4$								99·6	102·8			93·7					
$K(SbO)C_4H_4O_6$										97·8		97·4	95·3	76·2			
Fe_2Cl_6					95·4		78·5	53·1	0								
$FeSO_4 + 7H_2O$					92·0			63·7	47·6			46·9	39·2	36·1			
$MnCl_2$					100·3			101·6	94·4			66,7					
$ZnSO_4 + 7H_2O$					97·2		84·5	64·4	34·2								
$BaCl_2 + 2H_2O$								103·1				97·2				79·7	
$MgSO_4 + H_2O$								99·3				95·1				81·3	
$K_2Mn_2O_8$		99·8		99·1		95·0			87·1			35·2	0				
$K_2Cr_2O_7$								99·1		95·5		88·1	74·3	61·0			
KCN					98·3		97·6	99·8	100·9		125·2	126·7	115·4	105·6			
$K_4Fe(CN)_6 + 3H_2O$					95·4							96·4			89·8		
$K_6Fe_2(CN)_{12}$					96·8			95·3							87·5		
$Na_2B_4O_7 + 10H_2O$								106·5		114·5		130·3	138·3		141·7	131·2	116·5
$Na_2SO_4 + 10H_2O$								98·3				99·0			93·1		86·7
$KClO_3$								94·8				98·7			93·0		
KNO_3								99·3				98·5			94·1		87·8
KCl								98·7				99·8			89·4		83·2
NaCl								105·1		99·8		94·5	86·7		80·6	80·4	75·0
KBr								101·6				103·2			101·4		109·8
KI								105·0				99·4				99·7	
$(Mo)_2 . H_2SO_4 + 5H_2O$								98·7				96·2			89·1		
$(At)_2 . H_2SO_4$								99·4				96·6			87·8		
$(Na)_2 . H_2SO_4 + H_2O$								87·3				1·0			0		
$(Q)_2 . H_2SO_4 + 7H_2O$								97·5				69·3			45·1		
$(Ci)_2 . H_2SO_4 + 2H_2O$								96·4				67·4			31·4		
$(Cidine)_2 . H_2SO_4 + 3H_2O$								104·0				75·6			43·0		
$(Sr)_2 . H_2SO_4 + 6H_2O$								95·4				76·6			56·8		
$(Br)_2 . H_2SO_4 + H_2O$								92·6				82·3			82·8		

in the action of sodium tetraborate on the salivary ferment and on trypsin.

It is moreover, evident, from a comparison of the results obtained with the three ferments, that under the conditions of dilution, etc., with which our experiments were tried, the salivary ferment is the most sensitive to the action of the various salts, while of the other two, trypsin is as a rule most readily affected. Still, it is hardly possible to draw a direct comparison between the two proteolytic ferments, since they act under such different conditions; the fact that pepsin acts only in an acid medium and that both the strength and nature of the acid affect the activity of the ferment, introduces an additional factor which makes direct comparison in the case of the two ferments impossible. The possible reason for the slight acceleration of proteolytic action produced by several neutral salts, in the case of pepsin-hydrochloric acid, has been already referred to; but why neutral salts, which in large percentages show retarding action, should in smaller percentages added to a *neutral* solution of the ferment (ptyalin or trypsin) produce acceleration, can only be conjectured. We might assume a simple *stimulation* of ferment action by mere contact. That it may become very pronounced, is evident from the action of borax, potassium cyanide and potassium bromide in the case of trypsin and mercuric cyanide, ammonium arsenate, ferrous sulphate, potassium chlorate, sodium chloride, tartar emetic, and alkaloid salts in the case of the salivary ferment.

As to the manner in which the various salts produce retardation of ferment action, it would appear as if many of the results obtained, could be accounted for only by assuming, in addition to the views already offered, a direct influence in many cases, either destructive or hindering, on the ferment itself.

Those substances, which are particularly injurious to animal cells show in many cases no retarding action whatever, on the unformed ferments; this is particularly noticeable in the case of the arsenic compounds, which affect the ferments only as the solutions become acid. On the other hand, certain of the metallic salts are alike injurious to both and doubtless for the same reason, viz: on account of their power of combining with albuminous matter, which fact applies with equal force to the vegetable organisms (organized ferments). Nearly all germicides act injuriously on the unformed ferments. Many salts, however, well known as antiseptics, are without injurious action, except when present in large quantity; notably borax in the case of trypsin or boracic acid in the case of pepsin-hydrochloric acid.

Influence of Temperature on the Relative Amylolytic Action of Saliva and the Diastase of Malt. By R. H. Chittenden and W. E. Martin, Ph.B.

It has long been known that the ferment of saliva and the diastase of malt, differ from each other in the temperature best adapted to their amylolytic action. Paschutin* states that saliva ten times diluted, converts starch into dextrin and sugar most rapidly at 38° to 41° C., while the strongest action of the malt ferment occurs at 70° C.; rising slowly from 50° C. up to this temperature. According to Kühne,† the amylolytic action of salivary ptyalin is most energetic at 35° C., while the action of malt diastase is most rapid at 66° C.; ptyalin being destroyed at 60° C. Kjeldahl‡ states that the amylolytic power of diastase rapidly increases with increase of temperature up to +50° C. By 54° C., ferment action is more vigorous than at 50° C., while maximum action lies between +54° C. and +63° C. Above +63° C. amylolytic action rapidly diminishes with increase in temperature.

Exposing diastase for a long time to a temperature below 63° C. does not, according to Kjeldahl, weaken perceptibly the action of the ferment. Higher temperatures, however, cause a diminution in amylolytic power proportional to the length of time the ferment is heated; thus Kjeldahl states that long continued warming at +66° C. produces the same effect upon diastase as heating for a shorter time at +70° C. For the ptyalin of saliva, Kjeldahl finds the temperature most favorable for amylolytic action to be about +46° C. From this temperature, amylolytic action diminishes on both sides, although somewhat more rapidly by increase in temperature.

O'Sullivan§ and Brown and Heron‖ have also studied the influence of temperature on the amylolytic action of malt extract, more however with a view to ascertaining the relative proportion of products (maltose and dextrin) formed and the nature of the change involved, than any comparison of the effect of temperature on the energy of the ferment.

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* Quoted by Hoppe-Seyler, Physiologische Chemie, p. 187.

† Lehrbuch der Physiologischen Chemie, p. 20-21.

‡ Abstract in Jahresbericht für Thierchemie, 1879, p. 381-383.

§ On the action of malt-extract on starch, Journal Chem. Soc., 1876, ii, p. 125.

‖ Beiträge zur Geschichte der Stärke und der Verwandlungen derselben. Liebig's Annalen der Chemie, vol. cxcix, p. 213. Also Journal Chem. Soc.

Hüppe* likewise, has made a study of the effect of high temperatures on the two ferments, in manner similar to the experiments of Bull, Hüfner and Salkowski, not, however, to ascertain the effects of definite temperatures on ferment action, but rather to ascertain the extent to which the dry ferments can be heated without destroying their peculiar properties.

It has been our purpose to obtain by quantitative methods, definite expressions of the influence of temperature on the relative amylolytic action of the two ferments.

Our method of determining amylolytic action, is based upon the gravimetric determination of the cupric oxide-reducing power of the solution, resulting from the action of the ferment upon starch paste, according to the method of Allihn.† This gives very concise and definite results of admitted accuracy. The cupric oxide-reducing power of a solution, resulting from the amylolytic action of these two ferments, must necessarily express the degree of intensity of ferment action, since the more energetic the action, the larger the amount of sugar (maltose and dextrose) formed, with higher reducing power; while the weaker the action, the larger the amount of dextrins with lower reducing power.

The amylaceous material employed in the experiments, was purified corn starch. In each experiment 1 gram of the starch was made into a paste with 50 c. c. of boiling water, then 40 c. c. more water were added, and lastly, when everything was in readiness, 10 c. c. of the ferment solution, either saliva or malt extract; thus making a volume of 100 c. c. containing 1 per cent. of starch. In every case, the ferment was allowed to act upon the starch at the desired temperature for exactly thirty minutes, when further ferment action was at once stopped by the addition of a definite quantity of dilute acid. The ferment being thus destroyed, the solution was neutralized by adding an amount of sodium hydroxide equivalent to the acid, after which the solution was concentrated, then made up to exactly 100 c. c., and in 25 c. c., or one-fourth of the filtered fluid, the reducing bodies were determined. From the weight of metallic copper so obtained, the reducing bodies are, for the sake of comparison, calculated as dextrose, from which in turn is calculated the percentage of starch converted. Naturally the amount of starch digested, is larger than the figures indicate, since the reducing power of maltose and the dextrins is much smaller than that of dextrose, but the above method of calculation is most convenient and for comparison quite sufficient.

* Jahresbericht für Thierchemie, 1881, p. 446. Ueber das Verhalten ungeformter Fermente gegen hohe Temperaturen.

† Zeitschrift für Analytische Chemie, xxii, p. 448.

The saliva employed in the experiments was filtered, human mixed saliva, carefully neutralized and then diluted; 20 c. c. saliva to 80 c. c. water. As 10 c. c. of this fluid were used in each experiment, 1 gram of starch was exposed to the action of 2 c. c. of normal saliva in a dilution of 1:50.

The malt extract was prepared from coarsely ground, malted barley by extracting 10 grams with 200 c. c. water at 40° C. for 3–4 hours; then filtering, neutralizing and diluting to 500 c. c., a few drops of thymol being added to prevent acid fermentation. The amylolytic power of 10 c. c. of this diluted extract was a little more than that of a similar quantity of the dilute saliva. Each series of experiments, however, was made with different extracts, each of which showed considerable variation in amylolytic power.

A. *Amylolytic action at definite temperatures.*

In all of these experiments, the 90 c. c. of fluid containing the starch paste was brought to the desired temperature by immersion in a large water-bath carefully regulated, the thermometer being immersed in the vessel containing the starch paste. In a similar manner, the ferment solution was quickly brought to the same temperature, care being taken in the latter case, that the fluid did not go beyond the requisite point. When the temperature became constant, 10 c. c. of the ferment solution were added and the action continued for thirty minutes.

The results are clearly expressed in the following series of tables:

SERIES I.*—SALIVA.

Temperature.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
10° C.	0·0935 gram.	0·1904 gram.	17·19 per cent.
20	0·1227	0·2496	22·46
40	0·1410	0·2872	25·83
50	0·1003	0·2040	18·35

SERIES II.—SALIVA.

Temperature.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
20° C.	0·0891 gram.	0·1816 gram.	16·34 per cent.
30	0·0945	0·1924	17·31
40	0·1203	0·2448	22·03
50	0·1419	0·2888	25·99
55	0·1129	0·2296	20·66
60	0·0451	0·0936	8·42
65	0·0125	0·0282	2·53

* It is of course understood, that the results in any one series are obtained with the same ferment solution.

SERIES III.—SALIVA.

Temperature.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
20° C.	0·1177 gram.	0·2396 gram.	21·56 per cent.
30	0·1273	0·2592	23·32
40	0·1285	0·2616	23·54
45	0·1174	0·2392	21·52
50	0·1131	0·2300	20·70
55	0·1029	0·2092	18·82
60	0·0883	0·1800	16·16*
65	0·0354	0·0748	6·73
70	0·0017		

SERIES IV.—SALIVA.

Temperature.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
25° C.	0·0748 gram.	0·1528 gram.	13·75 per cent.
30	0·0897	0·1828	16·45
40	0·1070	0·2180	19·62
45	0·0990	0·2016	18.18
50	0·0806	0·1644	14·79
55	0·0715	0·1460	13.14
60	0·0278	0·0596	5·36
65	0·0142	0·0328	2·95

SERIES V.—SALIVA.

Temperature.	Wt. Cu in ½.	Total amount reducing bodies.	Starch. converted.
40° C.	0·1062 gram.	0·2164 gram.	19·47 per cent.
2	0·0611	0·1252	11·33

SERIES VI.—MALT EXTRACT.

Temperature.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
30° C.	0·1374 gram.	0·2800 gram.	25.20 per cent.
40	0·1520	0·3100	27·90
50	0·1606	0·3280	29·52
60	0·1408	0·2864	25·77
70	0·0544	0·1124	10·11
80	0·0127	0·0296	2·66

SERIES VII.—MALT EXTRACT.

Temperature.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
35° C.	0·1573 gram.	0·3208 gram.	28·87 per cent.
45	0·1552	0·3168	28·51
50	0·1575	0·3212	28·90
55	0·1617	0·3300	29.70
65	0·0585	0·1200	10·80
75	0·0366	0·0768	6·91

* See remarks further on, in regard to this high result.

SERIES VIII.—MALT EXTRACT.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
30° C.	0·1301 gram.	0·2648 gram.	23·83 per cent.
40	0·1471	0·2996	26·96
45	0·1542	0·3148	28·33
50	0·1488	0·3036	27·31
55	0·1320	0·2688	24·19
60	0·0691	0·1412	12·70
65	0·0654	0·1340	12·06

SERIES IX.—MALT EXTRACT.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
30° C.	0·1283 gram.	0·2612 gram.	23·50 per cent.
35	0·1435	0·2924	26·31
40	0·1507	0·3072	27·64
45	0·1562	0·3188	28·69
48½	0·1573	0·3208	28·87
50	0·1588	0·3244	29·19
55	0·1445	0·2944	26·45
60	0·0742	0·1516	13·64
65	0·0561	0·1152	10·36

SERIES X.—MALT EXTRACT.

Temperature.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
40° C.	0·1419 gram.	0·3042 gram.	27·36 per cent.
2	0·0299	0·0636	5·72

By a study of these results, it is evident that in the case of the salivary ferment, variations in amylolytic action are not very great between the temperatures of 20° and 50°, or even 55° C. With the temperatures experimented with, however, amylolytic action appears to reach its maximum, in the case of saliva, at 40°; although in one single instance, for some unaccountable reason it appeared to be greater at 50° C. With the diastase of malt on the other hand, amylolytic action reaches its maximum at 50° C., although in one instance it appeared somewhat greater at 55° C.; great variations, however, are not to be observed between the temperatures of 30° and 55° C. Brown and Heron,* working with extract of malt and pure potato starch at different temperatures, obtained results by determination of both specific rotary power and cupric oxide-reducing power, which point to the same conclusions as those obtained by us. Thus at 40° C., the malt extract having been previously heated at the same temperature for 20 minutes, these investigators found at the end of 30 minutes, as

* Liebig's Annalen der Chemie, vol. cxcix, p. 221. Also Journal Chem. Soc., 1879.

a result of the action of the malt extract on the starch $(a)j = 163{\cdot}3°$, while at 50° C. under the same conditions $(a)j = 162{\cdot}7°$ and at 60° C. $(a)j = 164{\cdot}1°$, or in a second experiment, $(a)j = 163{\cdot}7°$. These results show at 50° C. the formation of a little more maltose than at 40°, although the difference is very slight; while at 60° C. the amount of maltose formed, is less even than at 40° C. Evidently then, the maximum amylolytic action of diastase of malt takes place at temperatures far below 60° C.; even below 55° C.

At very low temperatures, there is a corresponding difference in the action of the two ferments, as is apparent from the results obtained at 2° C.; the ferment of saliva being comparatively far more active at this temperature than the ferment of malt.

Hence it is apparent throughout, that diastase requires a higher temperature than the salivary ferment, in order to act with equal vigor; at the same time it is evident that at the body temperature, say 40° C., the difference in action between the two ferments is not very great. At 80° C. the diastase of malt still acts upon starch, although only slightly; the salivary ferment, however, under the conditions of our experiments, does not act at all at 70° C. and only slightly at 65° C. With these higher temperatures, it makes considerable difference in the ultimate result, whether the ferment solution is quickly brought to the desired temperature or not, and whether it remains long at the temperature in question, before being added to the starch solution. Thus, in the action of saliva at 60° C., if the ferment be warmed quickly to *nearly* 60° C., say 59° C., and then added to the starch paste at 61° C., as was done in the case of Series III, amylolytic action is considerably greater than when the saliva is actually brought to 60° and kept there for a moment or so to be sure of its constancy. Some variation in the length of time, required to bring the ferment solution to the desired temperature, was unavoidable, and doubtless, slight variations in the results at higher temperatures, occur from this cause. It was not, however, our purpose at this time, to heat the ferment in order to induce a change in its character, but simply to prevent any alteration in the temperature of the starch mixture on addition of the ferment, so that the action of the ferment on the starch might take place at a constant temperature.

The effects, on amylolytic action, of exposing the ferment of saliva to different temperatures for varying lengths of time.

Brown and Heron* state that a malt extract, warmed quickly to 66° C. and then added at once to starch paste at the same temperature, differs but little, in the first stages of its action, from a malt extract heated at 60° C.; if, however, the malt extract be warmed for say 10 or 15 minutes at this temperature, previous to adding it to the starch paste, its amylolytic action is very much weakened. Evidently then, under the influence of the increased temperature, a portion of the ferment is destroyed or else changes are induced, by which the action of the ferment is modified. Results of like nature were previously obtained by O'Sullivan,† with malt extract.

With saliva, we have tried the following experiments, designed originally to throw light on the comparative destructibility of the ferment.

SERIES XI.—SALIVA.

The saliva was exposed to the designated temperature for the specified time, then added to the starch paste at the *same temperature* and its amylolytic power determined.

Temperature.	Time of exposure.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
60° C.	0 min.	0·0409 gram.	0·0852 gram.	7·66 per cent.
60	15	0·0213	0·0464	4·17
60	30	0·0210	0·0460	4·14

At 60° C. therefore, the coagulating point of albumin and the temperature at which ptyalin is supposed to be destroyed, it is apparent that destruction of the ferment is not complete even by 30 minutes exposure to this temperature. The peculiarity of the results, moreover, make it doubtful whether we have to do with destruction at all. If the reduced amylolytic action is due to simple destruction of the ferment, we should expect less ferment action after 30 minutes exposure than after 15 minutes; as it is, the action in the two cases is the same. A certain time, however, is required to produce the change in the character of the ferment. Similar results are shown in the following series of experiments, conducted in the same manner as the preceding, only at different temperatures.

* Loc. cit., p. 227.

† Loc. cit., p. 143.

SERIES XII.—SALIVA.

Temperature.	Time of exposure.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
50° C.	15 min.	0·0773 gram.	0·1576 gram.	14·18 per cent.
50	60	0·0765	0·1560	14·04
55	30	0·0474	0·0984	8·85
55	60	0·0414	0·0864	7·21

Comparing these results with those obtained at like temperatures in Series I.–III., it is seen that a few minutes exposure at the designated temperature, lowers materially the amylolytic power of the solution, while doubling the time of exposure does not materially affect the result; a fact which is not consistent with the view that diminution in amylolytic power, under these conditions, is due to gradual destruction of the ferment.

The following series of experiments, also with saliva, throw additional light on the action of high temperatures on this ferment. In these two series, the saliva was exposed to the designated temperature for the specified time, then *cooled to* 40° C. and added to the starch paste *at a like temperature.*

SERIES XIII.—SALIVA.

Temperature.	Time of exposure.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
40° C.	0 min.	0·1081 gram.	0·2200 gram.	19·80 per cent.
50	30	0·1026	0·2088	18·79
55	30	0·0986	0·2008	18·07
60	30	0·0279	0·0596	5·36

SERIES XIV.—SALIVA.

Temperature.	Time of exposure.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
40° C.	0 min.	0·1062 gram.	0·2164 gram.	19·47 per cent.
55	180	0·0798	0·1628	14·65

It would appear from these results, that by exposure of the saliva to 50° or 55° C., in the latter case for even 3 hours, and then cooling to 40° C. and testing the amylolytic power of the ferment at that temperature, less diminution of ferment action is to be observed. This speaks still more strongly against destructive action, by simple coagulation, and at the same time suggests that not only does exposure to say 55° C. affect the character of the ferment, but also that the action of the ferment so treated, is in a given time different at that same temperature from what it is at 40° C., or the temperature of maximum action. This latter point, however, which is contrary to the law laid down by Brown and Heron* for malt extracts at tem-

* Liebig's Annalen der Chemie, vol. cxcix, p. 221. Also, Journal Chem. Soc., 1879.

peratures above 50° C. we reserve for further investigation. Owing to the great difficulty in rendering saliva perfectly neutral, it is possible that the observed low result at 55° C. in Series XII. may be due to the presence of a trace of either acid or alkaline carbonate.

Finally, it is to be observed that the majority of the results obtained, indicate that the influence of different temperatures, on the amylolytic action of the salivary ferment, is due rather to change in the character of the ferment, than to the direct influence of the various degrees of heat upon the cleavage of the starch molecule; similar in character to that indicated by the work of O'Sullivan, and also of Brown and Heron in the case of malt diastase.

Influence of Bile, Bile Salts and Bile Acids on Amylolytic and Proteolytic Action.* By R. H. Chittenden and Geo. W. Cummins, Ph.B.

The influence of bile and bile acids on the digestive processes of the intestinal canal has long been considered an important one, still few experiments have been made to determine the exact influence of these substances by themselves on ferment action. The form in which the main constituents of the bile exist in the intestinal canal depends naturally upon the reaction of the contents of the intestines. If these have an acid reaction, bile acids must be present; if alkaline, salts of these acids; and it is fair to presume that under these two conditions the presence of bile may be productive of different effects on ferment action. Recorded observations tend to show that ordinarily the contents of the intestines possess a distinct acid reaction; thus Schmidt-Mülheim† has found that in dogs fed on albuminous matter, the contents of the small intestines are invariably acid, although the mucous membrane sometimes possesses an alkaline reaction. It is evident that in such cases the alkali of the bile must have combined with the acid of the chyme, which would be followed by liberation of the bile acids and partial precipitation of the same in combination with the proteid matters of the chyme. Moreover, the recorded observations of Schmidt-Mülheim tend to show that this acid condition of the contents of the intestines persists throughout the entire length of the intestinal canal. Uffelmann‡ has likewise found, in corroboration of the above, that the faeces of infants naturally nourished possess a weak acid reaction, while, on the other hand, Nothnagel,§ as a result of 800 observations, finds that human excrement, in the case of adults, varies decidedly in its reaction, being generally alkaline, more rarely acid or neutral. It is hardly proper, therefore, to conclude that it is only necessary to study the

* Also published in the American Chemical Journal, vol. vii, p. 36.

† Archiv für Physiologie, DuBois Reymond, 1879, p. 56.

‡ Jahresbericht für Thierchemie, 1881, p. 305.

§ Jahresbericht für Thierchemie, 1881, p. 309.

influence of the bile acids in their free condition on ferment action, since in the passage of the ferments through the intestinal canal there are times, doubtless, when the reaction of the mass is more or less alkaline, especially in the small intestines, for some distance beyond the opening of the bile and pancreatic ducts. In either case it is an interesting point to ascertain whether the bile salts have an action at all analogous in kind or extent to that of the free acids.

Many observations* are recorded concerning the duodenal precipitate formed in the duodenum by the action of bile on the acid-reacting chyme. The precipitate itself has generally been supposed to consist of a mixture of syntonin, peptone and bile acids, but recent experiments of Maly and Emich† with pure bile acids tend to show that only the non-peptonised albuminous bodies are precipitated, viz: coagulable albumin and syntonin, and these only by taurocholic acid, while peptone and "propeptone" remain in solution. This fact lends favor to the view advanced by Hammarsten, that the object of the precipitation of albuminous matter on the walls of the intestines is to prevent its too rapid passage through the intestinal canal, thus giving ample opportunity for the action of the pancreatic juice.

The addition of taurocholic acid to a solution of peptone, Maly and Emich find, is followed by the formation of a distinct opalescence or fine dust-like precipitate, slowly changing to fine droplets. This precipitate, however, which is doubtless the same as observed by Hammarsten and Brücke on the addition of bile to portions of a digestive mixture, does not contain according to Maly and Emich, any peptone, but consists of taurocholic acid, possibly in a modified form.

Both of these precipitations, however, would tend to mechanically throw down, to a greater or less extent, any ferment present, and thus diminish ferment action; but, as Maly points out, the main reason for a diminished action, in the case of pepsin, is to be sought for, not in a precipitation of the ferment, but in the formation of a compound of albumin with the bile acid, not digestible by pepsin-hydrochloric acid. But since this precipitation, as a normal reaction in the animal body, must take place in the intestinal canal, it is equally important to ascertain the extent of its digestibility in pancreatic juice, or, in other words, to ascertain the exact influence of bile and its several constituents on the proteolytic action of trypsin

* See Maly in Hermann's Handbuch der Physiologie, vol. v, p. 180.

† Monatshefte für Chemie, vol. iv, p. 89.

as well as on the action of pepsin and on amylolytic action. The only data bearing on these points are the recent experiments of Maly and Emich, who have found that 0·2 per cent. taurocholic acid hinders the digestive action of pepsin-hydrochloric acid, while 1 per cent. of glycocholic acid is without influence. The same investigators likewise state that 0·1 per cent. taurocholic or glycocholic acid stops the amylolytic action of the pancreas ferment, and that 0·2 per cent. taurocholic acid or 1 per cent. glycocholic acid will completely stop the amylolytic action of the salivary ferment.

Our experiments on this subject were commenced before the above results were published, and we have continued them, since we wished to ascertain likewise the influence of the bile salts, and also the effects of both salts and acids, as well as the bile itself, on the proteolytic ferment of the pancreas. The results of Maly and Emich, moreover, not being quantitative, do not express the relative effects of the various percentages of bile acids used, but simply the percentage of acid necessary to stop ferment action under the conditions described by them.

1.—*Influence on Amylolytic Action.*

As amylolytic ferment, we have employed filtered human mixed saliva made neutral and then diluted to a known volume. In studying the influence of the various percentages of bile salts and acids on the action of the ferment, we have used a digestive mixture (50 or 100 cc.) containing 1 per cent. of starch previously boiled with water, and 2 per cent. of saliva, together with the given percentages of bile salts or acids. The extent of diastatic or amylolytic action under the varying conditions was determined in each case by estimating the amount of reducing substances, maltose and dextrose, formed during 30 minutes warming at 40° C. Further diastatic action was at once stopped by boiling the digestive mixtures, after which they were diluted to a known volume, and the reducing substances determined in a given portion of the diluted fluid by Allihn's gravimetric method.* The reducing substances are in each instance calculated as dextrose, and the diastatic action is expressed in the percentage of starch converted into sugar.

We first tried the influence of crystallized ox bile, since bile itself contains a small amount of a diastatic ferment. A 1 per cent. solution of nicely crystallized ox bile was made, with which the following results were obtained:

* Zeitschrift für analytische Chemie, xxii, 448.

Crystallized bile.	Wt. Cu in ⅛.*	Total amount reducing bodies.	Starch converted.
0 per cent.	0·0643 gram.	0·2636 gram.	23·72 per cent.
0·01	0·0630	0·2584	23·25
0·02	0·0686	0·2804	25·23
0·03	0·0693	0·2836	25·52
0·05	0·0656	0·2688	24·19
0·10	0·0734	0·3000	27·00
0·20	0·0665	0·2724	24·51
0·35	0·0447	0·1860	16·74

Here it is plain that a mixture of sodium glycocholate and taurocholate, in such proportion as they are contained in crystallized ox bile, exerts no appreciable retarding influence on amylolytic action until present to the extent of 0·35 per cent. On the contrary, smaller percentages unmistakably tend to increase the diastatic action of the ferment. The solution of crystallized bile had, however, a slight acid reaction, and possibly this may have had some influence in giving the latter results. The saliva and starch were both neutral.

Experiments were next tried with sodium taurocholate alone, and also with sodium glycocholate. Following are the results:

Sodium taurocholate.	Wt. Cu in ⅛.	Total amount reducing bodies.	Starch converted.
0 per cent.	0·0787 gram.	0·3212 gram.	28·90 per cent.
0·3	0·0030	0·0146	1·51
0·5	0·0023	0·0112	1·00
Sodium glycocholate.			
0·5	0·0783	0·3196	28·76

It is thus plainly evident that sodium taurocholate has a very decided action on the amylolytic ferment of saliva, while the same percentage of glycocholate is entirely without effect. The retarding action of crystallized bile is thus, without a doubt, due wholly to the taurocholate. Moreover, even smaller percentages of sodium taurocholate retard amylolytic action with almost equal energy.

The following results were obtained under like conditions as the preceding, except that the 2 per cent. of saliva employed was not neutralized.

Sodium taurocholate.	Wt. Cu in ¼.	Total amount reducing bodies.	Starch converted.
0 per cent.	0·0590 gram.	0·1212 gram.	21·81 per cent.
0·14	0·0079	0·0192	3·45
Sodium glycocholate.			
0·20	0·0758	0·1548	27·86

* One-eighth of the entire digestive mixture.

Thus even 0·14 per cent. of sodium taurocholate under these conditions almost entirely stops amylolytic action. The smaller percentage of glycocholate, however, causes the same increased amylolytic action observed with the smaller percentages of crystallized bile.

With the bile acids the following results were obtained. The glycocholic acid used was a nicely crystallized specimen prepared from ox bile, while the taurocholic acid, prepared from the same source, was amorphous:

Per cent. bile acid.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·0694 gram.	0·1420 gram.	25·56 per cent.
0·01 taurocholic.	0·0753	0·1538	27·68
0·05	0·0783	0·1598	28·76
0·10	0·0060	0·0146	2·63
0·20	0		
0·05 glycocholic.	0·0523	0·1082	19·47
0·10	0·0095	0·0234	4·21
0·20	0·0056	0·0136	2·44
0·50	trace		
1·00	0		

It is thus seen that 0·1 per cent. taurocholic acid prevents amylolytic action almost entirely, while 0·2 per cent. does not allow the conversion of any starch into sugar. This agrees exactly with the results obtained by Maly and Emich.* These same investigators, however, found only a trace of amylolytic action in the presence of 0·05 per cent. taurocholic acid; a result which does not agree with what we have found, working, however, under somewhat different conditions.

The presence of 1·0 per cent. glycocholic acid entirely prevents the conversion of starch into sugar, while 0·5 per cent. allows only the smallest amount of diastatic activity. Maly and Emich likewise found that 1·0 per cent. of glycocholic acid stopped the diastatic action of saliva.

We have repeated the last series of experiments in part, using, however, normally alkaline saliva instead of neutralized.

Per cent. bile acid.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0	0·0590 gram.	0·1212 gram.	21·81 per cent.
0·1 glycocholic.	0·0107	0·0258	4·64
0·2	0·0057	0·0139	2·50
0·1 taurocholic.	0·0052	0·0126	2·26

* Loc. cit., p. 118.

These results agree exactly with the preceding, and both together plainly show that only small percentages of bile acids are required to entirely prevent the amylolytic action of saliva. Assuming that the amylolytic ferment of the pancreatic juice is similar in its nature to the ferment of saliva, it would follow from our experiments that whether the contents of the intestines are acid or alkaline, the presence, beyond a certain percentage, of taurocholic acid, either as free acid or as a taurocholate, would tend to diminish amylolytic action. Very small percentages, however, would have little, if any, retarding effect, indeed might increase amylolytic action. As to glycocholic acid, the free acid is much more powerful in its action on the amylolytic ferment than the sodium salt of the acid.

Considering these results in the light of a possible application to changes in the intestinal canal, it becomes an interesting point to ascertain whether bile itself exerts the same influence on amylolytic action as the bile salts. Moriggia and Battistini * state that while bile mixed with chyme gives a precipitate which, among other things, contains mucin, bile acids and pepsin, thus hindering gastric digestion, it does not, on being mixed with saliva, hinder its amylolytic action. This they found to be the case both with bile containing mucin and with bile from which the mucin had been removed by acidifying. We have, therefore, made the following experiments with fresh ox bile containing 7·46 per cent. of solid matter. The digestive mixtures contained as before 1 per cent. of starch, 2 per cent. of neutral saliva, and were warmed at 40° C. for 30 minutes:

Ox bile.	Wt. Cu in ½.	Total amount reducing bodies.	Starch converted.
0 per cent.	0·0753 gram.	0·3072 gram.	27·64 per cent.
2·0	0·0875	0·3568	32·11
5·0	0·0690	0·2824	25·41
10·0	0·0719	0·2944	26·50
20·0	0·0770	0·3144	28·30

Here in close accord with what has been found before, the presence of a small percentage of bile causes increased amylolytic action; larger percentages, however, have little, if any, effect; certainly not such an effect as would be expected from the known action of the bile salts. The bile itself possessed to a slight extent, diastatic action; 20 c. c. of the bile (20 per cent.) converting 4·53 per cent. of the starch into sugar in 30 minutes. This, however, could hardly account for the increased amylolytic action noticed above in the pres-

* Jahresbericht für Thierchemie, 1876, p. 196.

ence of 2 per cent. of bile. Wittich* and also Hofmann have noticed the occasional diastatic action of bile, Wittich even extracting the ferment from human bile by his glycerine method. Gianuzzi and Bufalini† have shown that the action varies considerably in bile from different animals and individuals, and without any apparent dependence upon the nature of the food. Ewald‡ states that the diastatic capacity of bile appears to be slight in all cases, and is not found in bile which has stood for some time. We have found, however, in bile from several animals considerable diastatic power; thus in one sample of fresh sheep's bile, 25 c. c. (25 per cent.) converted 24·33 per cent. of starch into sugar in 30 minutes at 40° C. We have likewise found great variation in diastatic power, varying, expressed in the percentage of starch converted into sugar under the conditions described, from 4 to 24 in the case of herbivorous animals. We have also noticed in bile from sheep and oxen the presence of a small amount of sugar, or at least a substance capable of reducing Fehling's solution. In one instance the amount was not inconsiderable; 25 grams of ox bile yielding, by Allihn's method, 0·040 gram metallic copper, equal to 0·0209 gram dextrose or 0·08 per cent. Naunyn, we believe, has already claimed the presence of sugar in bile.

While we know then that bile acids and bile salts by themselves retard very decidedly the amylolytic action of ptyalin, it would appear that the retarding influence of the latter may be, in part at least, counteracted by other substances naturally present in the bile.

2.—*Influence on the Proteolytic Action of Pepsin.*

It has long been known that bile has a retarding action on pepsin digestion, and Maly and Emich have recently shown the percentages of bile acids necessary to bring the action of pepsin to a standstill. We have, however, in addition, experimented with bile itself, and as in the case of the amylolytic ferment, have endeavored to study the influence of the bile acids quantitatively. The method employed for measuring proteolytic action is one frequently used in this laboratory, and which has invariably given satisfactory results. The only feature which calls for description is the preparation of the proteid matter to be digested. The material consists of carefully selected

* Jahresbericht für Thierchemie, 1872, p. 243.

† Jahresbericht für Thierchemie, 1876, p. 197.

‡ Lectures on digestion, Amer. ed., p. 77.

and thoroughly washed blood fibrin. All soluble matters are removed by successive extraction with boiling water, cold and boiling alcohol, and finally with cold and warm ether. The fibrin is thus obtained in a perfectly friable condition and can be easily ground to a coarse powder. It is then dried at 100–110° C. This material is well adapted for quantitative experiments with pepsin-hydrochloric acid; the residue remaining after a digestion can be rapidly filtered with the aid of a pump, and can be easily freed, by washing, from peptones and other soluble products of digestion.

The gastric juice employed in the experiments, consisted of a hydrochloric acid solution of a glycerine extract of the mucous membrane from a pig's stomach, in the proportion of 10 grams glycerine extract to 1 litre of 0·2 per cent. hydrochloric acid. 50 or 100 c. c. of this pepsin-hydrochloric acid were employed in each experiment, to which was added 1 or 2 grams of the dried fibrin (2 per cent.), together with the given percentage of bile or bile acids.

We first tried the influence of bile itself, using fresh ox bile, slightly alkaline in reaction and containing 10·02 per cent. of solid matter.

The digestive mixtures were warmed at 40° C. for two hours, then filtered at once, and the undigested residue washed thoroughly,* and dried at 100° C. until of constant weight. Following are the results of the first series of experiments, with 2 grams of fibrin and 100 c. c. of gastric juice.

Bile in digestive mixture.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·1957 gram.	90·21 per cent.
0·25	0·1890	90·55
0·50	0·2050	89·75
1·00	0·2234	88·83
3·00	0·5453	72·73
5·00	0·7642	61·84

* In all of the pepsin-hydrochloric acid digestions the presence of bile or bile salts naturally causes more or less of a precipitate, dependent in amount upon the percentage of bile and also upon the amount of digestive products. In washing the undigested fibrin it was of course necessary to remove this precipitate. This was accomplished by pouring over the precipitate on the filter 50 c. c. of 0·5 per cent. potassium hydroxide and then washing with water until the alkali was wholly removed.

The following experiment shows that under these conditions the alkali affects the swollen fibrin but little, if any. Two portions of fibrin of 2 grams each were warmed with 100 c. c. of 0·2 per cent. HCl for 30 minutes, then filtered and one washed with water alone, the other with water and alkali. The first gave 1·9272 grams dried residue, the other 1·9155 grams.

A second series, tried under the same conditions, but with larger percentages of bile gave the following results:

Bile in digestive mixture.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·1979 gram.	90·10 per cent.
0·25	0·2456	87·72
0·50	0·1927	90·36
9·00	1·1955	40·22
13·00	1·6611	16·94
16·50	1·7812	10·94
20·00	1·9241	3 29

From these two series of experiments it is evident that the presence of bile, from 1 per cent. upward, causes diminished proteolytic action, the retarding effect being proportionate to the amount of bile present. 20 per cent. of bile stops the action, under these conditions, almost completely. It is fair to presume, therefore, that the reflux of but a small amount of bile into the stomach would be productive of a diminished proteolytic action.

These results, therefore, agree with the older statements of Brücke, Hammarsten and others, to the effect that bile added to a gastric digestion has the effect of bringing the proteolytic action to a standstill. We next tried the influence of the individual bile acids with the following results:

Taurocholic acid.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·1311 gram.	86·89 per cent.
0·025	0·1461	85·39
0·050	0·2200	78·00
0·100	0·2421	75·79
0·200	0·2668	73·32
0·500	0·3579	64·21

Here it is seen that the smallest percentage of taurocholic acid added, produces a distinct effect on proteolytic action, and in the next series of experiments still smaller percentages of acid cause an equally marked effect. In both series of experiments, the mixtures were warmed at 40° C. for 1 hour and 30 minutes.

Taurocholic acid.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·1499 gram.	85·01 per cent.
0·010	0·1819	81·81
0·015	0·1900	81·00
0·020	0·2947	70·53
0·050	0·3110	68·90

Adding taurocholic acid to the digestive mixture in the form of a sodium salt has the effect of diminishing still further the action

of the ferment; doubtless, due in part to the percentage of free hydrochloric acid being diminished by decomposition of the taurocholate.

Taurocholic acid.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·2059 gram.	79·41 per cent.
0·1	0·6198	38·02
0·2	0·6426	35·74
0·5	0·6475	35·25

Maly and Emich found that 0·2 per cent. taurocholic acid entirely stopped the action of pepsin; in our experiments, however, ferment action was still manifest even in the presence of 0·5 per cent. of the acid. Whether this difference in result is due to difference in the acid used, or to difference in method, we cannot say. Glycocholic acid we found to be entirely without influence on the action of pepsin, as did also Maly and Emich.

3.—*The Proteolytic Action of Trypsin in Neutral, Alkaline and Acid Solutions.*

The trypsin solution was prepared according to Kühne's method,* from dried pancreas freed from fat; the solution after neutralization always contained some sodium salicylate, sufficient to prevent putrefaction during short digestive periods. According to Kühne,† trypsin acts quite energetically, both in neutral and in salicylic acid solutions, but most energetically when the pancreatic solution contains 0·3 per cent. sodium carbonate. According to Heidenhain,‡ the action of definite percentages of sodium carbonate varies with the amount of ferment.

We have tried quantitative experiments as a preliminary to studying the influence of bile, with the following results;§ the mixtures were warmed at 40° C. for 3 hours and 40 minutes, and contained 2 per cent. of fibrin.

Reaction of the fluid.	Weight of undigested residue.	Fibrin digested.
neutral	0·2312 gram.	76.88 per cent.
0·1 per cent. Na_2CO_3	0·1570	84·30
0 2	0·0925	90·75
0·3	0·0772	92·28
0·4	0·0426	95·74
0·5	0·1038	89·62
0·1 pr. ct. salicylic acid	0·5651	43·49

* Untersuchungen aus der physiolog. Inst. d. Universität Heidelberg, vol. i, p. 222.

† Ibid, p. 223.

‡ Pflüger's Archiv, vol. x, p. 576.

§ The pancreatic juice was prepared from 20 grams dry pancreas, and finally diluted to 1000 c. c. 50 c. c. were used in each digestion with 1 gram of pure fibrin.

With a larger percentage of fibrin and a longer period of digestion the results are somewhat different. The following were obtained with 4 per cent. of fibrin in 6 hours and 40 minutes at 40° C.:

Reaction of the fluid.	Weight of undigested residue.	Fibrin digested.
neutral	0·3785 gram.	62·15 per cent.
0·1 per cent. Na_2CO_3	0·2581	74·19
0·2	0·1395	86·05
0·3	0·1588	84·12
0·4	0·1629	83·71
0·5	0·1318	86·82
0·1 pr. ct. salicylic acid	0·4728	52·72

An average of the two series of results plainly shows that there is but little difference in digestive action in the presence of 0·2–0·5 per cent. sodium carbonate, although in a given solution a change in the percentage of alkali is at once manifest, to a slight extent, in the amount of fibrin digested. Greatly increased percentages of alkaline carbonate materially diminish the action of the ferment, as the following series of experiments indicate; the mixtures were warmed for 2 hours at 40° C.:

Reaction of the fluid.	Weight of undigested residue.	Fibrin digested.
neutral	0·5863 gram.	41·37 per cent.
0·5 per cent. Na_2CO_3	0·1584	84·16
1·0	0·3760	62·40
2·0	0·7010	29·90
3·0	0·7892	21·08
4·0	0·8373	16·27
5·0	0·8608	13·92

The difference in action between a neutral trypsin solution and a solution containing salicylic acid is quite noticeable, at the same time it is evident that in the acid-reacting fluid the ferment simply acts more slowly, and if time be given, the action will approach more closely to that of the neutral solution. It is of course understood that the salicylic acid in the above experiments does not exist in a free state, but in combination with the proteid matter present, and doubtless in most of the experiments recorded, where trypsin has been exposed to the action of small fractions of a per cent. of acid, no free acid has been present, but only varying percentages of acid-proteids.* Kühne† has pointed out that hydrochloric acid above 0·05 per cent. is injurious to the action of trypsin, and Heidenhain‡ has

* See Danilewsky. Centralbl. med. Wiss., 1880.

† Verh. Naturhist. med. Vereins zu Heidelberg, 1877, p. 193.

‡ Pflüger's Archiv, vol. x, p. 578.

shown that the addition of 0·1 per cent. hydrochloric acid to an aqueous extract of the pancreas stops its action. C. A. Ewald* however, found that while pepsin-hydrochloric acid destroyed trypsin, trypsin could digest fibrin in the presence of 0·3 per cent. hydrochloric acid. Mays† likewise found that trypsin digestion could take place in the presence of 0·3 per cent. hydrochloric acid, but only when a relatively large proportion of fibrin was present, and in corroboration of Kühne's statement, he showed that trypsin could be destroyed both by pepsin and dilute hydrochloric acid. Engesser‡ found that a pancreatic juice did not lose its tryptic power by two hours warming with a gastric juice containing 0·5 per cent. hydrochloric acid. Langley,§ on the contrary, has shown that a glycerine extract of the pancreas loses a very appreciable amount of trypsin when warmed for two and a half hours with 0·05 per cent. hydrochloric acid. Lindberger,‖ working with an alcohol precipitate from a glycerine extract of ox pancreas, in which there would naturally be present but a small amount of proteid matter in addition to the trypsin, found that in the presence of 0·1 per cent. hydrochloric acid the ferment was entirely without action, and even in the presence of 0·012 per cent. hydrochloric acid, fibrin was much more slowly dissolved than by a neutral trypsin solution. Lindberger, moreover, found that weaker acids, as acetic and lactic, had a much different effect than the stronger hydrochloric acid; thus with dilute acetic acid, digestion of the fibrin was almost as rapid as with a neutral solution of trypsin, while with small amounts of lactic acid, ferment action was even more energetic than in a neutral solution. There is, however, no guarantee that in these experiments free acid was present.¶

We have found that *free* acids, even when present in small percentages, completely stop the proteolytic action of trypsin, and that when considerable albuminous matter is present, the action of trypsin is much hindered by the addition of acid to a neutral solution, even before the proteid matters present are saturated with acid. 0·1 per cent. *free* salicylic acid, in the presence of proteids already satu-

* Jahresbericht für Thierchemie, 1880, p. 297.

† Untersuchungen a. d. physiolog. Inst. d. Univ. Heidelberg, vol. iii, p. 378, 1880.

‡ Jahresbericht für Thierchemie, 1880, p. 297.

§ Journal of Physiology, vol. iii, No. 3.

‖ Jahresbericht für Thierchemie, 1883, p. 281.

¶ We have seen only the abstract of Lindberger's paper, so cannot speak positively on this point.

rated with the acid, allows no proteolytic action whatever. Furthermore, a sufficient amount of proteid matter just saturated with hydrochloric acid, no free acid being present, will almost completely stop the action of trypsin. Proteid matter, however, only partially saturated with acid has a much smaller retarding action. This, doubtless, was the condition of the mixtures in Mays' and Engesser's experiments above referred to, for, as Mays states, the ferment could act in the presence of 0·3 per cent. hydrochloric acid only when a relatively large proportion of fibrin was present.

A pancreatic juice prepared from 20 grams of dried pancreas by warming it at 40° C. with 200 c.c. 0·1 per cent. salicylic acid, etc., was finally made exactly neutral and diluted to 500 c.c.; 25 c.c. of this solution required 7·5 c.c. of a 2·0 per cent. solution of salicylic acid to completely saturate the proteids present,* the excess of free acid necessary to give the tropæolin reaction being deducted.

Three digestive mixtures were made as follows:

1. 25 c.c. of the neutral pancreatic solution + 50 c.c. water.

2. 25 c.c. of the same pancreatic solution + 7·5 c.c. 2·0 per cent. salicylic acid solution + 17·5 c.c. water. The mixture was acid to test papers, but gave no reaction with tropæolin 00. It therefore contained no free acid, but 0·3 per cent. of combined acid.

3. The same as No. 2, but 2·5 c.c. more of 2·0 per cent. salicylic acid, so that the solution contained, in addition to the acid proteids, 0·1 per cent. free salicylic acid.

One gram of fibrin was added to each of these and the mixtures warmed at 40° C. for 8 hours and 40 minutes. No. 1 digested 88·34 per cent. of the fibrin, No. 2, 13·44 per cent., while No. 3 had no action whatever.

Much smaller percentages of combined salicylic acid cause an equally diminished proteolytic action; thus, in the case of a carefully dialyzed juice where the proteid matter was much diminished, the digestive mixture, with its proteids wholly saturated, contained but 0·060 per cent. of combined salicylic acid; yet this mixture, in 15 hours at 40° C. digested but 17·10 per cent. of fibrin, while the same amount of the neutral trypsin solution digested 57·80 per cent.

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* Tested by tropæolin 00 according to the method of Danilewsky (Centralbl. med. Wiss., 1880). One drop of a solution containing 0·028 per cent. free salicylic acid gives a reddish-violet color, which is, however, not permanent as in the case of hydrochloric acid, but transient. With hydrochloric acid, one drop of a 0·003 per cent. solution will give the reaction.

Combined hydrochloric acid has a greater hindering action than salicylic acid, as the following results show:

Pancreatic solution of trypsin.	Fibrin digested in 18 hours.
neutral	57·80 per cent.
0·034 per cent. combined HCl + no free HCl	3·90
0·034 " " HCl + 0·005 per cent. free HCl	2·31
0·034 " " HCl + 0·010 " "	0·87

It is thus evident that in an ordinary digestive mixture, or even where albuminous matter is present only in limited quantity, the addition of hydrochloric or salicylic acid to a neutral solution of trypsin reduces its proteolytic action to a minimum before any free acid is present.

4.—*Influence of Bile, Bile Salts and Bile Acids on the Proteolytic Action of Trypsin.*

The addition of bile to a *neutral* pancreatic juice causes but little change in its proteolytic action, as is seen from the following results obtained with ox bile containing 8·3 per cent. solid matter:

Bile.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·4118 gram.	59·82 per cent.
1·0	0·3907	60·93
10·0	0·3938	60·62

A slightly increased action is the only effect produced on the trypsin.* The addition of bile to an *alkaline* pancreatic juice does not produce any very different results. The following were obtained with a pancreatic juice containing 0·3 per cent. sodium carbonate and fresh ox bile containing 10·02 per cent. solid matter:

Bile.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·3056 gram.	69·44 per cent.
0·25	0·3074	69·26
0·50	0·3488	65·12
1·00	0·3633	63·67
5·00	0·3278	67·22
10·00	0·3603	63·97

Here there is no increased proteolytic action, neither is there any very great retarding effect produced. Pure sodium glycocholate and taurocholate produce results similar to bile, as the following table shows. The pancreatic juice contained 0·3 per cent. sodium carbonate:

* Compare Heidenhain, Pflüger's Archiv., vol. x, p. 579.

Sodium taurocholate.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·2308 gram.	76·92 per cent.
0·05	0·2566	74·34
0·10	0·3048	69·52
1·00	0·2832	71·68
sodium glycocholate.		
0·10	0·2576	74·24
0·20	0·3154	68·46

The presence of 3·0 per cent. crystallized ox bile caused a somewhat different result, increasing the proteolytic action slightly; thus, while the control, containing 0·3 per cent. sodium carbonate, digested 88·69 per cent. of fibrin, the same trypsin solution plus 3 per cent. of crystallized bile digested in the same time 89·73 per cent. of fibrin.

While bile or bile salts have but little influence on the proteolytic action of trypsin, the bile acids, even small percentages, have a much more marked effect. The following results, obtained by the addition of the bile acids to a neutral pancreatic juice, show the extent of the action:

Bile acids.	Weight of undigested residue.	Fibrin digested.
0	0·2516 gram.	74·84 per cent.
Glycocholic, 0·03 per cent.	0·1993	80·07
Taurocholic, 0·10	0·3455	65·45
0·20	0·4332	56·68
0·50	0·4170	58·30

Here the retarding influence of taurocholic acid is very manifest, while, on the other hand, the small percentage of glycocholic acid appears to increase the action of the ferment.

In view of the possible acid-reacting character of the contents of the small intestines, it becomes an interesting point to ascertain the influence of bile on the action of trypsin in the presence of more or less combined acid. With a pancreatic juice in which the proteids were partially saturated with salicylic acid, 0·1 per cent. combined acid being present, the following results were obtained:

Bile.	Weight of undigested residue.	Fibrin digested.
0 per cent.	0·4322 gram.	51·78 per cent.
1·0	0·4858	51·42
10·0	0·4091	59·09

This increased action in the presence of 10 per cent. of bile accords with Lindberger's results, this experimenter having found that bile in the presence of small percentages of (combined?) acetic and lactic acids tends to diminish the retarding effect produced by the acids alone.

In the presence of combined hydrochloric acid, the bile salts produced no effects whatever; the trypsin was entirely without action.

Absorption of Arsenic by the Brain. By R. H. Chittenden and Herbert E. Smith, M.D.

Some time since one of us* advanced the view that the amount of arsenic present in the brain, in cases of arsenical poisoning, is an index to the *form* in which the poison was taken, viz: whether in a readily soluble and diffusible form, such as sodium arsenite, or in a comparatively insoluble form, as arsenious oxide or aceto-arsenite of copper. The original experiments of Scolosuboff† on animals, with sodium arsenite, plainly showed the capability of nerve tissue for the absorption of arsenic; yet the recorded observations of toxicologists tend to show, as a rule, the presence of but traces of this metal in cases of arsenic poisoning, either acute or chronic. Scolosuboff's results are, however, undoubtedly correct; arsenic when taken in a very soluble and diffusible form without doubt does accumulate in the brain, but in our opinion *only when in that condition*, and thus in the more common forms of poisoning with the white oxide or other insoluble forms of arsenic, but a trace of the poison is to be found in the brain at any one time.

With a firm belief in the truth of the above statement, founded on personal experience and the recorded results of other workers in this field, it was maintained by one of us in a previous paper‡ that the presence of weighable amounts of absorbed arsenic in the brain may be taken as an indication of the administration of a soluble form of the poison. Experiments on animals tend to show the correctness of the theory and the results of toxical investigations, so far as our knowledge extends, contain nothing contrary to this view. If true, we ought never to find under any circumstances an accumulation of arsenic in the brain, after the administration of an insoluble form of the poison. Hence, the study of arsenic cases, where the form of poison is known, is of great importance in this connection.

We have had a recent opportunity of adding two more cases to

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* Chittenden, Amer. Chem. Jour., vol. v, p. 8; and Medico-Legal Journal, vol. ii, p. 237.

† Bulletin de la Société Chimique de Paris, vol. xxiv, p. 125.

‡ Chittenden, loc. cit.

the list of those which bear testimony to the truth of the above theory; two fatal cases of arsenic poisoning, one of which was caused by the white oxide, the other presumably by Paris green or aceto-arsenite of copper.

Case A.—L. K., a middle-aged laboring man, ate for his dinner at noon a quantity of bean soup. Almost immediately after, he was seized with vomiting and purging, cramps in the legs, and all the ordinary symptoms of acute arsenic poisoning. There were no marked cerebral symptoms. At 9 P. M. of the same day the patient died in a condition of collapse, having thus lived nine hours after eating the poisoned soup. An autopsy made the following day by Dr. M. C. White of the Yale Medical School, to whose courtesy we are indebted for a description of the case, and also for the organs for analysis, revealed the following points of interest: "The mucous membrane of the stomach was very much inflamed, especially around the cardiac orifice. The duodenum was likewise much inflamed, also the lower part of the rectum, showing here as a red mottled congestion. The remaining portions of the intestines were normal. The brain showed marked congestion. The kidneys were normal in appearance, the urinary bladder was nearly empty, and the mucous lining somewhat reddened. The lungs were normal, except the lower half of the right one, which was a little congested. The heart normal; small fibrinous clot in the right ventricle." In order to draw deductions of any value from the distribution of arsenic in the body of the deceased we must know positively as to the form in which the poison was taken. Fortunately, we were able to obtain the residue of the soup eaten by the deceased. Microscopic examination of the sediment plainly showed octahedral crystals of arsenious oxide, and we were able to separate from a small portion of the solid residue 24 milligrams of the oxide. Plainly the soup was poisoned by simply mixing with it arsenious oxide in substance. As to the quantity of arsenic present in the soup we have the following data: 125 c. c., oxidized with hydrochloric acid and potassium chlorate, yielded 314·6 milligrams of arsenious sulphide, equal to 253·2 milligrams of arsenious oxide. As to the amount taken by the deceased we have no knowledge; we infer, however, since the soup constituted the main portion of his dinner, that a large quantity was eaten, which view we think is substantiated by the intensity of the vomiting and purging so characteristic of large doses of the poison.

Here then, we have an unquestionable case of poisoning with arsenious oxide, and under conditions most suitable for rapid absorp-

tion; a probable empty condition of the stomach, together with a large amount of the poison, a considerable portion of which must probably have been dissolved in the soup. Added to this, nine hours intervened between the taking of the poison and death. Certainly then everything favored an absorption of poison by the brain, if such is characteristic of this form of arsenic. Naturally the vomiting and purging would remove much of the poison, still the relative proportion of absorbed arsenic would not be materially altered, and thus if Scolosuboff's results with soluble arsenites are applicable to arsenious oxide, we ought to find in this case, in conformity with his results, a larger percentage of arsenic in the brain than in the liver or kidneys.

Following are the results actually found:*

Liver (1259 grams) contained 76·0 milligrams As_2O_3.
Kidney and bladder (332 grams) contained 0·6 milligram As_2O_3.
Brain ($\frac{1}{2}$=328 grams dry) contained simply a recognizable trace.

Case B.—J. G., a young woman, age unknown. Regarding the details of this case we have less definite knowledge. She was last seen alive on Friday night, at which time she threatened to poison herself. The following Monday morning she was found dead, and near her an open package of *Paris Green.* She had evidently been dead some time, and both the condition of her room and person gave evidence of excessive purging and vomiting. An autopsy by Dr. White showed an entire absence of inflammation of the alimentary tract, and also a lack of any abnormal condition sufficient to account for death. The verdict was therefore, death by poisoning with Paris green or aceto-arsenite of copper.

Through the kindness of Dr. White we were able to obtain portions of the body for analysis. The contents of the stomach were entirely free from arsenic, the poison having been wholly removed by the purging and vomiting; the trace found therefore, was the amount absorbed by the muscle tissue of the stomach. Following are the amounts of arsenic found in the parts analyzed:

Liver (2984 grams) contained 12·78 milligrams As_2O_3.
Kidneys and bladder (515 grams) contained 3·40 milligrams As_2O_3.
Muscle of thigh (735 grams) contained 0·97 milligram As_2O_3.
Stomach (425 grams) contained a trace.
Brain (1179 grams) contained a trace.

* The method of analysis consisted in the oxidation of the tissue with nitric and sulphuric acids, the arsenic being weighed as metallic arsenic. See Amer. Chem. Journal, vol. ii, p. 235.

The trace of arsenic in both the muscle tissue of the stomach and in the brain was very small; the entire brain could not have contained more than 0·2 of a milligram of arsenic.

These results plainly substantiate the views set forth above and lend favor to the belief that Scolosuboff's results with sodium arsenite are applicable only to that form of poison, and not to the more insoluble compounds of arsenic. These two cases, therefore, are additional evidence that in poisoning with arsenic the presence of an appreciable amount of poison in the brain, is an indication amounting almost to proof positive of the administration of a soluble and diffusible form of arsenic.

Influence of Potassium and Ammonium Bromides on Metabolism. By R. H. Chittenden and W. L. Culbert, Ph.B.

As a question in the physiology of nutrition, it is very desirable to be able to state something definite regarding the influence of bromides upon the metabolism of the body; particularly their influence upon the metabolism of proteid matter, as shown in the excretion of urea and uric acid, and in view of their special application as therapeutic agents in diseases of the nervous system, their influence also on the decomposition of nerve substance, as shown in the excretion of phosphoric acid. Two complete investigations appear to have been made upon this subject; one in 1868 by Dr. J. H. Bill,* and one in 1888, by Dr. B. Schulze,† the results of which are more or less in direct opposition to each other. We have also seen a reference to two other investigations, quoted by Dr. Wood,‡ in which Dr. Rabuteau found the daily excretion of urea slightly lessened under the influence of bromide, as did also Dr. Bartholow.

Dr. Bill's investigation, which was a very thorough one, had for one of its objects to ascertain whether bromides reduce the amount of phosphoric acid excreted, like such known hypnotics as morphine; at the same time careful examination was made of the variations in urea and uric acid under the different conditions of the experiments. The experiments were all conducted on one person with a body weight of 160 pounds, which remained fairly constant throughout. The experiments were made in series under known conditions with uniformity of habits, diet, etc. Unfortunately, however, no data are given regarding the nature of the diet, the closeness with which it was adhered to, or whether the body was kept in a state of nitrogenous equilibrium. Each series of experiments, moreover, covers at the most, but six days; three days without bromide and three days with, consequently slight variations might easily be absorbed in an average of three results. The urea in Dr. Bill's work was deter-

* Amer. Jour. med. Sciences, July, 1868. Experimental Researches into the action and Therapeutic value of Bromide of Potassium.

† Zeitschrift für Biologie, vol. xix, p. 301. Einfluss des Bromkalium auf den Stoffwechsel.

‡ Therapeutics, p. 337.

mined with a "mercury solution," phosphoric acid with uranium solution and uric acid by precipitation and weighing as such. The results obtained were as follows: With moderate doses of potassium bromide (3·0–8·0 grams per day) urea was not affected, phosphoric acid was slightly increased, uric acid likewise, though much more decidedly; with larger doses of bromide (10·0–14·0 grams per day, continued for 2–3 days) phosphoric acid was diminished in amount, but this Dr. Bill intimates could not be attributed to regular hypnotic action, since the other urinary constituents were likewise diminished, notably the urea. Both large and small doses of bromide increased the quantity of urine passed in the twenty-four hours. This Dr. Bill asserts was not due to the increased drinking of water, for no thirst, not even with the largest doses, was ever present.

Dr. Schulze, experimenting on his own person, obtained results quite different from these. This investigator lived on a fixed diet of the following composition:

220 grams fresh meat	=7·13 grams N.
50 grams air-dried wheat bread	=0·92 grams N.
30 grams cocoa powder	=1·14 grams N.
30 grams butter.	
30 grams sugar.	
5 grams salt.	
1500 grams water.	
	9·19 grams N.

The average amount of nitrogen excreted daily was about 11 grams.

Taking this amount of food daily, with uniform habits of sleep, exercise, etc., Dr. Schulze states that he soon reached a point where the daily excretion of nitrogen, sulphur and phosphorus remained fairly constant. Potassium bromide was then taken three days, in divided doses of 10 grams each day. Diuretic action was very noticeable. Phosphorus was diminished, sulphur very much increased, and nitrogen (on two days) apparently slightly increased under the influence of the bromide. As, however, the increased excretion of sulphur was not accompanied with a corresponding increase in the excretion of nitrogen, Schultze considers that the increase in sulphur cannot be due to increased metabolism of simple albuminous matter, and seeks to show that the potassium bromide must have decomposed, to a slight extent, some nitrogenous phosphorized principle or principles, such as lecithin (glycerine-phosphoric acid) and nuclein, so abundant in the brain and nerve substance in general. Schulze therefore concludes that under the influ-

ence of potassium bromide there is probably a decided diminution of metabolic activity in the nervous system, accompanied by decreased nervous irritability.

By determining the nitrogen of the fæces, Schulze concluded that the bromide exercised no particular influence on the digestibility of the food.

In our experiments great care was taken first, to insure body equilibrium and then to obtain sufficient data by analysis, to be sure of the requisite constancy in the composition of the daily excretions. The experiments were tried throughout on the person of one of us (W. L. C.), of good physique and vigorous constitution. The diet was weighed out each day with scrupulous care and was as follows:

Fresh meat [beef]	142·0 grams.
Potatoes	283·5 "
Wheat bread	256·0 "
Oat meal	50·0 "
Butter	56·7 "
Sugar	28·3 "
Salt	0·7 "
Milk	700·0 "
Water	345·5 "

This diet was commenced on the third day of April and continued for nine days before any attempt was made to ascertain the daily amounts of urea, etc., excreted. Then the urine was analyzed for nine successive days, after which doses of potassium bromide were taken. The above daily amount of food was divided into three portions and taken at the same time each day; at 7:30 a. m., 1 p. m., and 6 p. m. Exercise was taken regularly and in stated amounts; consisting of a walk each morning before breakfast and exercise with dumbbells just before retiring at 11 p. m. Care was taken not to exercise so freely as to induce perspiration. Throughout the day routine duties allowed of regular habits. It was thus found possible to keep even the minor conditions of the experiment constant throughout. The urine was collected from 7:30 a. m. of one day to 7:30 a. m. of the next, and was at once analyzed. Urea was determined by Pflüger's* modification of Liebig's method, with a standard solution of mercuric nitrate. All the precautions so carefully worked out by Pflüger; preparation of a mercuric nitrate solution of the proper specific gravity, a standard solution of sodium carbonate to neutralize the acid of the former, preliminary determination of

* Pflüger's Archiv, vol. xxi, p. 248.

chlorine and removal of the same, before precipitating the urea; were carried out with very satisfactory results. Uric acid was determined according to the older method of Heintz* with the modification of Zablins, and being conducted each time under exactly the same conditions and with a urine of approximately the same composition, the results are to be considered as strictly comparable. Chlorine and bromine were determined in the usual manner with a standard solution of silver nitrate; the results, however, are not given as they are of value only as essential to the urea determinations. Total phosphoric acid was determined by means of a standard solution of uranium nitrate.† Phosphoric acid in combination with alkali-earths was determined by precipitation with ammonium hydroxide, allowing the mixture to stand 24 hours, filtering the precipitated phosphates, washing thoroughly with diluted ammonia, then dissolving in a definite amount of dilute acetic acid and titrating with uranium solution. Total amount of solid matter contained in the 24 hours' urine, was calculated by the use of Christison's formula.

The diet specified, was commenced on the 3d day of April; on the 12th the urine was collected for analysis, the body weight taken and the investigation then carried forward without interruption. Table No. I. gives the results of the analysis of the urine for nine consecutive days, and shows the average amount of variation to be expected under the conditions of the experiment.

On the 21st of April, 60 grains of potassium bromide were taken in divided doses as seen in Table No. II. The bromide was taken about midway between the hours of eating, so that it might not affect digestion. On the 22d the dose was increased to 100 grains and then to 150, the latter amount being taken daily for three consecutive days. Table No. II. shows the effects of the bromide on the system, for the six days it was taken.

On the first day, the only apparent influence of the bromide is to cause a diminution in the amount of phosphoric acid excreted; seen both in the total P_2O_5 and in the P_2O_5 in combination with alkali-earths. On the second day, the diuretic action of the salt is apparent, accompanied with an increase in specific gravity, and a decided increase in the amount of urea excreted, together with a slight increase in the amount of uric acid. Phosphoric acid was still diminished in amount. On the third day, the body weight commenced to diminish and continued to do so throughout the experiment; diuretic

* Die Lehre vom Harn, Salkowski und Leube, p. 94–95.

† Die Lehre vom Harn, p. 184.

action was still apparent as was also increased elimination of urea, and to a slight extent of uric acid likewise. Indeed, the most noticeable effect of the bromide, next to its diuretic action is its decided influence on proteid metabolism as shown by the increased elimination of urea. As to phosphoric acid the results are not so striking, although an average of the two series shows a diminished excretion, both of total P_2O_5 and of P_2O_5 in combination with alkali-earths. The average difference in the two series of results is clearly shown by the following table:

	Average of Table No. I, without KBr.	Table No. II, with KBr.
Total quantity of urine	926 c. c.	1010 c. c.
Sp. Gr.	1025, 8	1026, 3
Total solid matters	56·7329 grams.	63·6252 grams.
Total P_2O_5	2·7540	2·5426
P_2O_5 in combination with Ca and Mg	0·6022	0·5452
Uric acid	0·6752	0·6858
Urea	34·8681	35·9454

Our results therefore plainly indicate, that under the influence of potassium bromide, nitrogenous metabolism is increased while the excretion of phosphoric acid is slightly diminished in amount; not however, to any such extent as would be expected by an active hypnotic agent. The bromide taken, the largest doses about 10 grams per day, produced its usual physiological effects; such as drowsiness, diminution of the circulation with accompanying coldness and paleness of the skin. Constipation was not noticed while taking the bromide, but later on it became somewhat troublesome, once or twice alternating with a slight diarrhœa. In accord with Dr. Bill, we noticed an increase in the acidity of the urine while taking the bromide, as also a deepening of the color.

With bromide of potassium therefore, our results agree with those of Dr. Schulze in showing an increased excretion of nitrogen (urea and uric acid), although far more pronounced than he found in his experiments, while the diminution in phosphorus is less pronounced than found by Schulze.

With Dr. Bill's experiments our results agree in so far as the diminution of phosphoric acid is concerned, but are entirely different as regards the urea. Since, however, Dr. Bill retained uniformity in diet only during the days of the experiments, it is quite possible that lack of nitrogenous equilibrium may have had some influence on his results. The increased elimination of urea noticed in our experiments is certainly indicative of increased metabolic activity; it is,

however, suggestive that potassium bromide has been recently found to exercise a very decided accelerating influence on the proteolytic action of both pepsin-hydrochloric acid* and trypsin;† while, therefore, this fact may have something to do with the increased elimination of nitrogen, particularly as the diet used is quite rich in proteid matter, it seems more probable to suppose that the above changes in the excretion of nitrogen are due rather to changes in the tissue proteids; still it would have been interesting if the nitrogen in the fæces had been determined each day.

Although the last dose of potassium bromide was taken on the 26th of April, the same diet was still continued and the urine carefully examined daily, until the 8th of May, at which time the amount of bromine in the urine was reduced to a minimum. Dr. Bill states that bromine usually disappears entirely from the urine in ten days after the last dose of bromide.

The results of the twelve days analyses are shown in Table No. III. In examining this table it is interesting to note how quickly the elimination of urea is changed on stopping the doses of bromide. On the 26th, the last day the bromide was taken, the excretion of urea amounted to 37·5 grams; on the 27th it fell to 31·8 grams, far below what it had been any time before the bromide was taken. It would thus appear that after withdrawal of the bromide, nutrition which had been accelerated, rebounded in proportion to the preceding acceleration. Uric acid, moreover, which had likewise been increased in amount by the bromide, was now also correspondingly diminished. Furthermore, the diuretic action of the bromide was at once stopped, and the specific gravity fell to 125·5. In the case of phosphoric acid, however, the action of the bromide appears to be continued for a day or two after its withdrawal, and indeed it is noticeable throughout, that the diminution in phosphoric acid excreted, is not at all proportional to the amount of bromide taken. In fact phosphoric acid, both total P_2O_5 and alkali-earth P_2O_5, appears to be more decidedly diminished on those days when the amount of bromide in the blood was the smallest, notably on the 21st, 24th and 27th of April. By the 3d day after withdrawal of the bromide, the excretion of urea had gone nearly back to the daily amount, prior to taking the bromide; still it is to be seen in Table No. III, that the average excretion of urea, uric acid and phosphoric acid is below the average excretion recorded in Table No. I. In fact after the continued doses

* Chittenden and Allen. Trans. Conn. Acad., vol. vii.

† Chittenden and Cummins. Ibid.

of potassium bromide, the metabolism of the body did not fall back to its original height; but being temporarily accelerated during the exhibition of potassium bromide, it [the nitrogenous metabolism] fell back on withdrawal of the same, to a far lower level, and although later somewhat increased in amount, its average was still lower than recorded in Table No. I. Nutrition had evidently been disturbed; the body weight showed gradual diminution, and on the 4th and 6th of May there was slight diarrhœa accompanied with a decided decrease in the amount of urea and uric acid excreted.

Dr. Bill appears to have experimented somewhat with sodium bromide, although we find no results recorded, aside from the fact that this salt, like potassium bromide, caused an increased excretion of uric acid, and the general statement that "when taken by the mouth, bromide of sodium does not produce the same effects as bromide of potassium." In view of the increased excretion of urea, noticed under the influence of the potassium salt, we were interested in seeing whether ammonium bromide would have a like influence, especially in view of the fact that v. Schrœder* has shown that ammonium carbonate is directly convertible into urea by passage through the liver.

The physiological action of ammonium bromide is stated to resemble in many points that of potassium bromide, while in other points it differs essentially.† As to its influence on metabolism no experiments whatever appear to have been made.

On the 9th of May, 75 grains of ammonium bromide were taken, in divided doses, as shown in Table No. IV. In all, 425 grains of the salt were taken in four consecutive days. The action of the salt on the system was not as pleasant as that of potassium bromide; causing a general weakness and indisposition, a slight diminution in the pulse, an occasional cold perspiration, more marked lividity of the countenance and a parched, dry taste in the mouth. An habitual eruption of the skin was moreover much increased and accompanied with acne on the back and shoulders. Undoubtedly these disagreeable symptoms were much augmented by the temporary lassitude which was beginning to be apparent; doubtless due to the approach of warm weather together with lack of the accustomed vigorous exercise and the long continued use of the somewhat monotonous diet.

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* Archiv f. exp. Pathol., vol. xv, p. 364. Also Report on Progress in Physiological Chemistry in Amer. Chem. Jour., vol. v, p. 219.

† Wood, Therapeutics, p. 341.

TABLE I.—WITHOUT BROMIDE.

April.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.
	grams.				grams.	grams.	gram.	gram.	grams.
12	72100	950 c. c.	Acid.	1026	59·0473	2·7678	0·6576	0·7443	35·7596
13	72100	930	Acid.	1026	57·2590	2·8596	0·6132	0·6901	35·0067
14	72000	1012	Acid.	1025	60·4227	2·7249	0·6060	0·6654	34·3699
15	72000	920	Acid.	1025	54·9298	2·7088	0·5587	0·6969	35·3247
16	72800	928	Acid.	1025	55·4074	2·7352	0·6188	0·7146	35·4567
17	72600	915	Acid.	1025·5	55·7510	2·6818	0·5946	0·7407	32·5379
18	72400	880	Acid.	1026·5	55·7755	2·6909	0·5569	0·5936	34·5360
19	72100	972	Acid.	1026	60·4147	2·9227	0·6399	0·6551	36·4960
20	72100	830	Acid.	1026	51·5887	2·7002	0·5746	0·5769	34·3255

TABLE II.—WITH POTASSIUM BROMIDE.

April.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.	Amount of KBr taken, in grains.
	grams.				grams.	grams.	gram.	gram.	grams.	
21	72200	870 c.c.	Acid.	1026	54·0749	2·2169	0·5542	0·6895	34·7129	20 at 10 a. m. 20 3 p. m. 20 10 p. m.
22	72200	1005	Acid.	1027	64·9816	2·5268	0·5805	0·7075	36·0992	33 at 10 a. m. 33 3 p. m. 34 10 p. m.
23	71700	1018	Acid.	1027	65·7715	2·6630	0·5411	0·6388	36·1708	50 at 10 a. m. 50 3 p. m. 50 10 p. m.
24	71600	920	Acid.	1025·5	56·0557	2·2876	0·4805	0·6118	35·1002	50 at 10 a. m. 50 3 p. m.
25	71500	1125	Acid.	1026·5	71·3039	2·9013	0·5096	0·6750	36·4778	50 at 10 a. m. 50 3 p. m. 50 10½ p. m.
26	71000	1120	Acid.	1026	69·6137	2·6605	0·6056	0·7924	37·5117	50 at 10 a. m. 50 3 p. m. 50 10 p. m.

TABLE III.—WITHOUT BROMIDE.

April.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.
27	grams. 70800	880 c. c.	Acid.	1025·5	grams. 50·5720	grams. 2·5779	gram. 0·4527	gram. 0·6420	grams. 31·8277
28	70400	840	Acid.	1025·5	51·1813	2·5628	0·5008	0·6846	32·3742
29	70500	905	Acid.	1025·5	55·1417	2·7606	0·4810	0·7647	33·3858
30	70400	945	Acid.	1025·5	59·2743	2·5806	0·4996	0·6693	33·4907
May. 1	70400	985	Acid.	1024·5	57·6068	2·5676	0·5326	0·6368	34·3289
2	70500	1050	Acid.	1023·5	58·8438	2·5138	0·5435	0·6405	33·4344
3	70100	1020	Acid.	1024·5	59·6533	2·6682	0·5987	0·6324	33·8654
4	69800	885	Acid.	1025	49·8547	2·3155	0·4438	0·5716	30·6414
5	70100	905	Acid.	1024	51·8222	2·5849	0·4476	0·7858	33·1222
6	69600	905	Acid.	1023	49·6144	2·5682	0·4350	0·6149	31·9801
7	69600	830	Acid.	1026·5	52·6064	2·5625	0·4949	0·6536	34·2450
8	70000	930	Acid.	1025	56·5945	2·5102	0·5373	0·6738	31·5995

TABLE IV.—WITH AMMONIUM BROMIDE.

May.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.	Amount of $(NH_4)Br$ taken, in grains.
	grams.				grams.	grams.	gram.	gram.	grams.	
9	69700	1040 c. c.	Acid.	1024	59·5416	2·4514	0·4895	0·6630	34·2265	25 at 10 a. m. 25 3 p. m. 25 11 p. m.
10	69700	1130	Acid.	1024·5	66·0864	2·2248	0·6581	0·6690	33·3503	33 at 10 a. m. 33 3 p. m. 34 11 p. m.
11	70100	990	Acid.	1025	59·1092	2·5581	0·5470	0·6633	34·8876	40 at 2 p. m. 30 7 p. m. 30 11 p. m.
12	70400	1130	Acid.	1024	64·7062	2·8177	0·6051	0·7051	36·1377	50 at 2 p. m. 50 7 p. m. 50 11 p. m.

TABLE V.—WITHOUT BROMIDE.

May.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.
	grams.				grams.	grams.	gram.	gram.	grams.
13	70400	970 c. c.	Acid.	1024·5	56·7291	2·3746	0·4329	0·6936	33·5846
14	70400	885	Acid.	1025·5	50·8766	2·1505	0·4061	0·7156	31·2645

However this may be, the use of ammonium bromide in our experiments gave rise to more unpleasant symptoms than the use of like amounts of the corresponding potassium salt. Like the potassium salt, ammonium bromide caused increased acidity in the urine and a brighter color. As to its influence on metabolism, a study of Table No. IV, and comparison with No. III, plainly shows a decided accelerating influence on the excretion of urea; the average of the results, moreover, shows a very slight diminution in the excretion of total phosphoric acid, at the same time it would appear, in accord with what was observed with potassium bromide, that the diminution was greatest with the smallest amounts of bromide, as on May 9th and 10th and on the 13th and 14th after withdrawal of the bromide. With the largest amount of ammonium bromide, on the other hand, phosphoric acid appeared to be increased in amount, thus according with what Dr. Bill observed with like quantities of potassium bromide.

The average difference in the two series of results is shown by the following table:

	Average of Table No. III without $(NH_4)Br$.	No. IV with $(NH_4)Br$.
Total quantity of urine	915 c. c.	1072 c. c.
Sp. Gr.	1024, 8	1024, 4
Total solid matters	54·3970 grams.	62·3608 grams.
Total P_2O_5	2·5643	2·5130
P_2O_5 in combination with Ca and Mg	0·4972	0·5749
Uric acid	0·6599	0·6751
Urea	32·8579	34·6505

It is thus seen that diuretic action is even greater with the ammonium salt than with potassium bromide; likewise the excretion of both urea and uric acid is greater under the influence of ammonium bromide than in the case of the potassium salt; as to phosphoric acid the table of averages shows practically nothing, but as before observed a study of the individual results does indicate some action of the salt, although diminution in the excretion of phosphoric acid under the influence of ammonium bromide cannot be so surely claimed as with the potassium salt.

After withdrawal of the ammonium bromide, the urine was examined for two days more; the results showing the same, or even greater drop in the excretion of urea, observed under like conditions with the potassium salt (Table No. V.). Hence, so far as our experiments extend, the influence of the two salts on the metabolism of the body is very much alike, differing only in extent of action; the ammonium

salt, as might be expected, causing the largest excretion of urea, not, however, necessarily from any greater influence on proteid metabolism, but merely as furnishing a certain amount of ammonia to be excreted as urea. Finally, our results, with both salts, fail to show that diminution in the excretion of phosphoric acid to be expected from active hypnotic agents, and in this respect, therefore, our results show nothing antagonistic to Dr. Bill's conclusion "that bromide of potassium in its legitimate action, is an anæsthetic to the nerves of the mucous membranes and a depressor of their action. Its hypnotic effects are secondary."

Influence of Cinchonidine Sulphate on Metabolism. By R. H. Chittenden and Henry H. Whitehouse, Ph.B.

While much attention has been paid to the physiological study of the cinchona alkaloids; quinine particularly having been experimented with by several observers, to ascertain its influence on the metabolism of the body; we have not been able to find any recorded statements bearing on the action of the closely related alkaloid, cinchonidine. In physiological action, quinine is taken as a type of the group; cinchonine is stated* to be similar to quinine but less powerful, and that its history in the organism is parallel with that of quinine; cinchonidine is likewise stated to be weaker than quinine and in physiological action, apparently its equivalent when taken in doses one-third larger. Presumably, therefore, its action on the metabolism of the body is similar to that of quinine, although apparently no attempt has been made to determine this point. In view of the special action of quinine on nitrogenous metabolism, we have devoted our attention mainly to a study of the influence of cinchonidine on the excretion of urea, uric acid, phosphoric acid and chlorine. The experiments were conducted wholly on the person of one of us (H. H. W.) under uniform conditions of diet, exercise, etc. The diet, weighed out accurately each day, was composed of

255 grams meat (beef).
255 grams wheat bread.
149 grams potatoes.
50 grams oat meal.
35 grams butter.
21 grams sugar.
570 grams milk.
350 grams water.

This diet, divided into three definite portions each day, was taken for some time previous to the experiments, so that the system might become accustomed to it and the metabolism of the body brought to a constant point. The body weight was determined each morning; the urine collected from nine A. M. of one day to nine A. M. of the next, making the 24 hours' urine, the analysis being made the same

* H. C. Wood, Therapeutics, p. 81.

day. Urea was determined by Pflüger's * modification of Liebig's method, chlorine being removed by a standard solution of silver nitrate. Chlorine was determined by evaporating 10 c. c. of the urine with a weighed amount of potassium nitrate in a platinum crucible, igniting until the organic matter was completely removed, dissolving in water, acidifying with nitric acid, neutralizing with calcium carbonate and then titrating with silver nitrate solution. Uric acid was determined by Heintz's method as modified by Zablins† and phosphoric acid with a standard solution of uranium nitrate.‡ The amount of solid matter was calculated by the use of Christison's formula.

After taking the above diet for some time, the urine was analyzed for seven consecutive days prior to the exhibition of cinchonidine. The results, seen in Table No. I, show a very close agreement in the daily excretions.

On the 11th of May the first dose of cinchonidine sulphate was taken. The alkaloid salt was a finely crystallized preparation obtained from Powers and Weightman. The daily dose was usually divided into three portions and taken in tiny gelatine capsules, about five hours apart. In view of the fact that cinchonidine is a weaker alkaloid than quinine, it was not deemed necessary to try the influence of very small doses; on the first day, therefore, 15 grains of the salt were taken; on the second day 21·8 grains; on the third day 35·1 grains; and on the fourth 50 grains, making a total of 121·9 grains of cinchonidine sulphate in four consecutive days.§ The results of the analyses of the four days' urine, as well as those of the three following days, on which no cinchonidine was taken, are shown in Table No. II.

Comparing these results with those in Table No. I, and in Table No. III, it is seen that cinchonidine exercises a very decided influence on the nitrogenous metabolism of the body. Urea is at once affected: its excretion on the first day even, is diminished 6 per cent., while on the second day it is diminished 10 per cent., and on the fourth day when the largest dose of cinchonidine was taken, the excretion of urea was 16 per cent. less than in the normal urine. The influence

* Pflüger's Archiv, vol. xxi, p. 248.

† Die Lehre vom Harn, Salkowski and Leube, p. 94–95.

‡ Die Lehre vom Harn, p. 184.

§ While taking the larger doses of cinchonidine, an intense ringing in the ears was temporarily experienced (cinchonism) together with partial deafness and slight dizziness. On one or two occasions a slight nausea was felt.

TABLE I.—NORMAL URINE.

May.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Chlorine.	Total P_2O_5.	Uric acid.	Urea.
	grams.				grams.	grams.	grams.	gram.	grams.
4	59400	975 c. c.	Acid.	1027·5	64·191	6·082	2·586	0·774	40·801
5	59400	950	Acid.	1028	63·713	5·553	3·044	0·797	42·684
6	59200	905	Acid.	1027	58·471	5·493	2·708	0·721	41·877
7	59600	990	Acid.	1027	63·962	6·510	2·710	0·819	42·999
8	59600	1077	Acid.	1026·5	68·262	6·324	2·810	0·768	41·464
9	59600	1055	Acid.	1026·5	66·867	6·166	3·219	0·749	41·640
10	59600	1052	Acid.	1026	65·387	6·149	3·040	0·764	41·985

TABLE II.—SHOWING THE EFFECTS OF CINCHONIDINE.

May.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Chlorine.	Total P_2O_5.	Uric acid.	Urea.	Am't Cinchonidine sulphate taken.
	grams.				grams.	grams.	grams.	gram.	grams.	grains.
11	59600	920 c. c.	Acid.	1027	59·440	5·044	2·776	0·713	39·715	15
12	59900	975	Acid.	1026·5	61·797	5·886	2·693	0·779	37·967	21·8
13	60000	1195	Acid.	1024	68·428	7·435	2·529	0·792	38·322	35·1
14	60000	1022	Acid.	1024	58·522	5·855	2·128	0·575	35·115	50
15	60200	1030	Acid.	1023	56·467	6·725	1·795	0·598	35·481	0
16	59400	950	Acid.	1026·5	60·212	6·028	2·390	0·666	35·989	0
17	58800	975	Acid.	1027·5	64·191	5·586	2·801	0·711	39·856	0

TABLE III.—SHOWING THE EFFECTS OF CINCHONIDINE.

May.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Chlorine.	Total P_2O_5.	Uric acid.	Urea.	Am't Cinchonidine sulphate taken.
	grams.				grams.	grams.	grams.	gram.	grams.	grains.
18	59600	900 c. c.	Acid.	1028·5	61·468	4·879	3·227	0·701	40·279	0
19	59600	920	Acid.	1028·5	62·834	5·270	3·019	0·744	43·453	0
20	59000	950	Acid.	1029·5	67·225	5·735	3·109	0·893	43·354	0
21	59000	875	Acid.	1030	62·997	4·851	3·035	0·699	40·999	50
22	59500	1047	Acid.	1027·8	69·704	6·708	2·674	0·705	40·407	45·8

TABLE IV.—SHOWING AVERAGE RESULTS.

	Table I, Normal urine.	Table II, with Cinchonidine.	Table II, 3 days following the last dose of Cinchonidine.	Table III, without Cinchonidine.	Table III, with Cinchonidine.
Total quantity urine	1001 c. c.	1028 c. c.	985 c. c.	923 c. c.	961 c. c.
Sp. Gr.	1027	1025·4	1025·6	1028·8	1029
Total solid matters	64·408 grams.	62·047 grams.	60·290 grams.	63·842 grams.	66·351 grams.
Chlorine	6·040	6·055	6·113	5·295	5·780
Total P_2O_5	2·874	2·532	2·329	3·118	2·855
Uric acid	0·770	0·715	0·658	0·779	0·702
Urea	41·421	37·780	37·109	42·362	40·703

of cinchonidine on the elimination of urea continues to be felt after discontinuing the use of the alkaloid; thus even three days after the last dose of cinchonidine, the elimination of urea is 6 per cent. less than in the normal urine. On the fourth day it is nearly back to the normal amount [see Table No. III.], and on the fifth and sixth days the excretion of urea rises somewhat above the average. Uric acid does not appear to be correspondingly affected. It is only under the influence of the largest doses, or rather under the long continued action of the alkaloid that the excretion of uric acid is diminished; thus on May 14th, when the final dose of 50 grains of cinchonidine sulphate was taken, the excretion of uric acid was for the first time diminished; its diminution, however, was very perceptible and it continued for the two succeeding days, after withdrawal of the alkaloid salt. Phosphoric acid was greatly diminished in amount under the influence of cinchonidine; the diminution commencing to show, as in the case of urea, with the first dose of the alkaloid salt, then gradually increasing in amount with increase in the dose of cinchonidine until maximum diminution was reached on the day after the final dose of the alkaloid. The alkaloid salt, moreover, appears to have had a slight diuretic action.

On the 21st of May, cinchonidine sulphate was again taken, the results of the analyses of the 19th and 20th showing that the urine had returned to its normal composition. Accordingly, 95·8 grains of the salt were taken on the 21st and 22d, producing the same results (Table No. III.) as noticed in the first series, viz: diminution in the amount of urea and uric acid excreted, a like diminution in the amount of phosphoric acid and an increase in the total amount of fluid.

Table No. IV. shows the *average* results under the different conditions of the experiments.

It is evident then, that cinchonidine has the power of lessening very materially the elimination of nitrogen, i. e. the consumption of tissue.

Ranke* first pointed out the retarding influence of quinine on the elimination of uric acid. Zuntz found by experiment upon himself that 25 grains of quinine reduced his elimination of urea nearly 40 per cent. A like diminution in the excretion of urea, under the influence of quinine, was noticed by Rabuteau in experiments upon dogs and also by Hermann von Boeck.† The most interesting experiments, however, with quinine are those carried out by Dr. G.

* Quoted from Dr. H. C. Wood, Therapeutics, p. 74.

† Zeitschrift für Biologie, vol. vii, p. 422.

Kerner,* and more recently by Dr. Prior† and Dr. Sassetzky.‡ In Dr. Prior's article is to be found a very complete account of the literature of the subject. Dr. Kerner found that taking 9·3 grains of quinine hydrochloride per day in divided doses, for 3 days, making a total of 27·9 grains, caused a diminution in the excretion of urea, amounting on an average for the three days to 12 per cent., while the excretion of uric acid under like conditions was diminished 54 per cent. Phosphoric acid, too, was diminished somewhat; on an average about 4 per cent. per day. Diuretic action was very slight.

With larger doses of quinine; 77·5 grains of the hydrochloride in divided doses during three days; diuretic action was quite pronounced, the average increase in volume, amounting to 200 c.c. Moreover, urea was diminished on an average 23 per cent. per day, uric acid 82 per cent. and phosphoric acid 15 per cent. Oppenheim,§ however, by a dose of 30·8 grains of quinine found an increase in the daily excretion of urea amounting to 4 grams, and he considers that Kerner's results are due to diminution of digestive action. It is certain that both quinine and cinchonidine do interfere with the proteolytic action of the gastric and pancreatic juices,‖ but this retarding action can hardly be taken as explaining in full, the results obtained by Kerner or those obtained by us with cinchonidine.

Prior, moreover, by very carefully conducted experiments with quinine, has completely corroborated Kerner's results and has shown in addition, by a daily determination of nitrogen in the fæces, that diminution of urea and uric acid is not due to lack of digestive action, as suggested by Oppenheim. Prior's results show on an average, without reference to the size of the dose, the following effects of the quinine.¶

Quantity of urine.	Urea.	Uric acid.	Sodium chloride.	Sulphuric acid.	Phosphoric acid.
increase	decrease	decrease	decrease	decrease	decrease
10·65 per cent.	19·60 %	72·29 %	9·06 %	33·70 %	23·38 %

Sassetzky's results with fever patients, also corroborate Kerner's statements.

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* Pflüger's Archiv, vol. iii, p. 104.

† Ueber den Einfluss des Chinin auf den Stoffwechsel des gesunden Organismus. Pflüger's Archiv, vol. xxxiv, p. 237.

‡ Ueber den Einfluss fieberhafte Zustände und Antipyretischer Behandlung auf den Umsatz der stickstoffhaltigen Substanzen und die Assimilation stickstoff-haltiger Bestandtheile der Milch. Virchow's Archiv, vol. xciv, p. 485.

§ Pflüger's Archiv, vol. xxiii, p. 476–477.

‖ Chittenden and Allen; Chittenden and Cummins. Trans. Conn. Acad., vol. vii.

¶ Pflüger's Archiv, vol. xxxiv, p. 263.

v. Boeck considers that quinine owes its retarding influence on proteid metabolism to a direct action of the alkaloid upon the cells and their activity, although the alkaloid doubtless does unite with albumin or alter its constitution so as to render it less readily decomposable. Metallic salts, as lead and mercury, certainly form compounds with albumin difficultly decomposable, as also does arsenic, and v. Boeck suggests that these metallic poisons unite with the proteid matter of the various organs of the body, while quinine unites simply with the circulating albumin, explaining in this manner the ready elimination of quinine as compared with the slow excretion of mercury or arsenic, the latter of which v. Boeck* has shown has little if any influence on proteid metabolism. Prior, moreover, states that diminution in the urine of the end-products of nitrogenous metabolism is due, not to hindering of their excretion, but to actual hindering of their formation.

Comparing now Kerner's results, with the results obtained by us with cinchonidine, we see great similarity of action but decided difference in extent, particularly so far as the excretion of uric acid is concerned. With cinchonidine, the greatest average daily diminution in uric acid amounts to but 15 per cent., and this after taking about 121 grains of the alkaloid during four consecutive days. Selecting the lowest single result, that obtained on the day 50 grains of cinchonidine were taken and comparing the diminution then, with the average normal excretion, it is seen to amount to but 26 per cent. In the case of urea and phosphoric acid, the divergence is not so great; thus for urea the average daily diminution was 11 per cent. for the three days following the last dose of cinchonidine, while the greatest diminution noticed in any one day was 16 per cent.; with phosphoric acid the average diminution for the same period amounted to 19 per cent., while the greatest diminution noticed any one day was 38 per cent.; a diminution which at no time was reached in Kerner's experiments with quinine.

Thus in drawing a comparison between Kerner's results with 77·5 grains of quinine distributed through three days, and our results with 121 grains of cinchonidine extended over four days, we see two striking points of difference; with quinine there is a diminution in the amount of uric acid excreted of 82 per cent., with cinchonidine an average diminution of but 15 per cent.; with quinine there is a diminution of phosphoric acid amounting to 15 per cent., with cinchonidine, on the other hand, a diminution of 19 per cent. Hence it is to

* Zeitschrift für Biologie, vol. vii, p. 430,

be seen that cinchonidine has a far less pronounced specific action on the excretion of uric acid than quinine, while on the other hand, diminution of phosphoric acid is much more pronounced with cinchonidine than with quinine. In Prior's experiments, however, with quinine, diminution of phosphoric acid is more pronounced.

Is this diminution in the excretion of phosphoric acid under the influence of cinchonidine to be attributed simply to decrease of proteid metabolism, or is it in part due to a special action of cinchonidine on the metabolism of some phosphorized principles, presumably those of nerve tissue? If due to general decrease of proteid metabolism, we might expect to find that the addition of any non-nitrogenous principle to our fixed diet, whereby the decomposition of albuminous matter would be diminished, would cause a corresponding decrease in the excretion of phosphoric acid, or in other words that diminution of urea and phosphoric acid excreted, would be in the same ratio as noticed under the influence of cinchonidine.

This question we have endeavored to answer by a study of the influence of pure glucose on the elimination of urea, uric acid, and phosphoric acid, under the same conditions of diet etc., as observed in the experiments with cinchonidine.

The influence of carbohydrate food on proteid metabolism has been illustrated in many ways by various investigators, but so far as we know, no experiments with pure glucose have ever been tried. Through the courtesy of Dr. Arno Behr, of Chicago, we have been supplied with an abundance of chemically pure anhydrous glucose, which we have used in the following experiment. Before taking the sugar, the urine was analyzed for ten consecutive days, to insure an accurate average of the normal excretion under the conditions of the experiment. The results are shown in Table No. V.

200 grams of glucose were then taken daily in addition to the fixed diet, for nine consecutive days. The effect on the excretion of urea, etc., is shown in Table No. VI. At no time was sugar to be detected in the urine by Trommer's test. A comparison of the two tables shows the usual effects of carbohydrate matter on the excretion of nitrogen, viz: a diminution in the amount of both urea and uric acid. The volume of the fluid excreted, appears to be considerably lessened by taking the glucose. The excretion of phosphoric acid is likewise diminished.

TABLE V.—NORMAL URINE.

Day of month.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Chlorine.	Total P_2O_5.	Uric acid.	Urea.
	grams.				grams.	grams.	grams.	grams.	grams.
1	59400	1045 c. c.	Acid.	1029	72·658	6·636	3·220	1·060	46·284
2	59600	1060	Acid.	1028·5	72·395	7·030	3·249	1·068	46·468
3	59400	1070	Acid.	1028	71·761	6·194	3·333	0·802	46·607
4	59200	1042	Acid.	1029	72·450	6·090	3·167	0·907	45·770
5	60000	960	Acid.	1028	64·384	6·258	2·886	0·928	43·161
6	59800	990	Acid.	1028·5	67·615	6·399	2·659	0·804	44·078
7	59800	1004	Acid.	1026	62·404	6·320	2·621	0·722	44·000
8	59800	1090	Acid.	1027	70·423	7·780	3·304	0·715	47·103
9	59400	1073	Acid.	1027·5	70·643	8·141	3·045	0·921	45·900
10	59400	970	Acid.	1028	65·045	6·978	2·597	....	45·390

TABLE VI.—SHOWING THE EFFECTS OF GLUCOSE.

Day of month.	Body weight.	Total quantity Urine.	Reaction.	Sp. Gr.	Total solid matters.	Chlorine.	Total P_2O_5.	Uric acid.	Urea.	Am't of Glucose taken.
	grams.				grams.	grams.	grams.	gram.	grams.	grams.
11	59600	935 c. c.	Acid.	1028·5	63·858	7·200	2·700	0·664	43·588	200
12	59500	900	Acid.	1030	64·797	6·727	2·781	0·721	42·114	200
13	59300	865	Acid.	1029	60·143	6·028	2·731	0·693	40·778	200
14	59800	840	Acid.	1028·5	57·370	4·862	2·440	0·663	40·009	200
15	59900	970	Acid.	1028·5	66·249	6·433	2·916	0·795	40·070	200
16	60000	1047	Acid.	1028	70·219	7·120	3·130	0·850	42·502	200
17	59600	950	Acid.	1028	63·713	6·674	2·832	0·642	40·887	200
18	59800	970	Acid.	1028	65·055	6·106	2·654	0·787	39·800	200
19	60000	965	Acid.	1026·5	61·163	5·640	2·527	0·684	38·752	200
20	60200	1072	Acid.	1026	66·630	7·290	2·934	0·846	41·740	0
21	59600	1014	Acid.	1026	63·025	6·553	2·655	0·809	39·885	0
22	60000	1076	Acid.	1026	66·879	6·289	3·108	0·901	45·439	0
23	59000	1056	Acid.	1026	65·636	6·350	2·962	0·891	44·492	0

These points of difference are all shown more clearly in the following table of averages :

	Average of Normal urine.	Urine under the influence of glucose.
Total quantity urine	1030 c. c.	938 c. c.
Sp. gr.	1027·9	1028
Total solid matters	68·97 grams.	63·62 grams.
Chlorine	6·78	6·31
Total P_2O_5	3·00	2·75
Uric acid	0·88	0·72
Urea	45·47	40·94

Coming now to the point at issue, viz : the relation between the amounts of urea and phosphoric acid excreted, we find that under the influence of glucose the average diminution of urea amounts to 10 per cent., while the average diminution of phosphoric acid under the same conditions is 8·34 per cent.

With cinchonidine, on the other hand, the average diminution of urea amounts to but 8·8 per cent., while the average diminution of phosphoric acid under like conditions is 11·9 per cent. Or, if we take the average of the three days following the last dose of cinchonidine, when both urea and phosphoric acid reach their maximum diminution, and compare these results with the average of the normal excretion we see that while the diminution of urea amounts to 10·4 per cent., the average diminution of phosphoric acid is raised to 18·97 per cent. Consequently, it would appear that while cinchonidine lowers the rate of decomposition of proteid matter in the body, it also has an effect upon the decomposition of some phosphorized principles, that being the only plausible explanation of the increased diminution of phosphoric acid noticed under the influence of the cinchonidine salt.

The Post-mortem Formation of Sugar in the Liver, in the presence of Peptones. By R. H. Chittenden and Alexander Lambert, B.A., Ph.B.

Claude Bernard's discovery in 1848, that the liver contains sugar, both before and after death, led at once to the inquiry as to the source of the sugar. This was apparently answered by Bernard's later discovery of glycogen, an amylaceous body readily convertible into sugar by acids and various ferments. Thus, Bernard's theory that the liver sugar resulted exclusively from glycogen has long been an accepted fact. In 1880, however, Seegen and Kratschmer in the first of a series of investigations,* state that the sugar formed in the liver does not have its origin, as supposed by Bernard, *wholly* in glycogen but that it is undoubtedly formed *in part* from other material. In a later communication† the same investigators show, in corroboration of their previous statement, 1. that the amount of sugar in the liver is increased very rapidly after death, in one case nearly 50 per cent. of the entire amount being formed within 10 minutes, while the whole process comes to an end inside of 24 hours; 2. that the glycogen formed in the liver is much more resistant to ferment action than has hitherto been supposed and that consequently the post-mortem formation of sugar by the action of a ferment upon glycogen could not take place so rapidly as the above. Moreover, direct experiments with dogs and with rabbits showed that in the first few hours after death, there was but little if any diminution in the amount of glycogen. Hence, Seegen and Kratschmer claim that the amount of glycogen remaining essentially the same, while the amount of sugar is greatly increased, tends to show conclusively that the liver sugar must be formed from some other material than glycogen and they venture the opinion that this source, whatever it may be, furnishes all of the liver sugar.

Boehm and Hoffmann,‡ however, take exception to the views of Seegen and Kratschmer, claiming possible analytical inaccuracies from the methods of procedure. They show, moreover, by experi-

* Ueber Zuckerbildung in der Leber. Pflüger's Archiv, vol. xxii, p. 236.

† Pflüger's Archiv, vol. xxiv, p. 467.

‡ Ueber die postmortale Zuckerbildung in der Leber. Pflüger's Archiv, vol. xxiii, p. 205.

ments on cats and dogs, that after death, contrary to the statements of Seegen, increased formation of sugar is attended with a corresponding decrease of glycogen, at least within such limits as are incident to the errors of experiment; further that in the case of a cat's liver 32 per cent. of the liver glycogen disappeared in 24 hours after death, thus indicating less resistance to the action of ferments than would be implied by Seegen's and Kratschmer's results.

In a later investigation,* Seegen shows that pieces of finely divided liver, kept in contact for an hour or longer with a solution of peptone yield a larger amount of sugar and even of total carbohydrates, than equal weights of the same liver under like conditions of treatment, without peptones. These results were obtained with the livers of calves, rabbits and dogs. Seegen, therefore, concludes that the liver is capable of forming from peptones, sugar and carbohydrates which are convertible into sugar.

A study of the analytical data plainly shows that the increase in sugar and total carbohydrates in the presence of peptone, although pronounced, is not great. The following experiment† with a calf's liver obtained from the market shows the most marked increase.

		With peptone.		Without peptone.	
No.	Time of the experiment.	Sugar.	Total carbohydrates.	Sugar.	Total carbohydrates.
I.	30 minutes	3·84 %	9·52 %	3·40 %	8·8 %
II.	48 hours	3·56	8·92	3·70	8·6
III.	96 "	2·66	8·00	2·82	7·8

Here the increase in total carbohydrates is seen to be only 0·72 per cent. and of sugar only 0·44 per cent. after 30 minutes. In Nos. II and III, longer standing in contact with the peptone tends to reduce the amount of sugar and to diminish the increase of total carbohydrates. This is attended with increase of acidity and Seegen considers that a portion of the sugar is decomposed in this long contact with peptone with formation of acid.

In a still later communication,‡ Seegen reports the results of other experiments tending to confirm his theory of the formation of carbohydrate matter from peptones in the liver. Thus, by feeding peptones to dogs, Seegen found that the content of sugar in the livers of eight dogs was considerably greater that in the normal liver, taking for the latter value the average of a number of determinations.

* Die Einwirkung der Leber auf Pepton. Pflüger's Archiv, vol. xxv, p. 165.

† Pflüger's Archiv, vol. xxv, p. 171.

‡ Pepton als Material für Zuckerbildung in der Leber. Pflüger's Archiv, vol. xxviii, p. 99.

Likewise, by the injection of peptone solutions directly into the portal circulation of dogs, Seegen found the amount of sugar in the liver increased two and even nearly three times above the normal amount. Lastly, by warming portions of freshly excised liver at 40° C., with a solution of peptone in water and some fresh, defibrinated blood, through which a constant current of air was made to pass, the amount of both sugar and total carbohydrates was considerably greater than under like conditions, but without peptones. The following experiment* taken from Seegen's account, illustrates the average increase of carbohydrates under this method of treatment.

Two portions of a dog's liver taken 15 minutes after death, were mixed with 50 c. c. of water and 50 c. c. of defibrinated blood. To one portion 5 grams of peptone were added and air passed through the mixture for 5 hours. Following are the results obtained in both:

Wt. of portion of liver.	Method of treatment.	Liver sugar.	Total carbohydrates.	Glycogen.
40 grams.	without peptone and blood,	3·04 %	6·9 %	2·12 %
40 "	with peptone and blood,	3·87	8·4	2·02

Other experiments indicated that peptones themselves are without diastatic action and that the blood and air (to form oxyhaemoglobin) are by themselves without influence on the liver. Hence Seegen concludes that the liver cells, retained in a living condition by the action of blood rendered arterial by a current of air, are capable of forming from peptone more or less sugar; thus establishing, if true, that the animal organism is able to form carbohydrates from albuminous material.

This is certainly a very important question, for if Seegen's views are correct they overthrow the long accepted belief in the origin of liver sugar in the hepatic glycogen. It is true that Bernard himself, before his discovery of glycogen, thought that the liver sugar originated in albumin and there have always been, up to the present time, difficulties in explaining the origin of liver carbohydrates on the dehydration theory alone. As is well known, a certain amount of glycogen is formed during a purely animal diet and in chronic cases of Diabetes the excretion of sugar is continued even on a pure albuminious diet. Moreover, the suggestion has been before made that peptones in their passage through the liver undergo change. Thus Plosz and Gyergyai† noticed that while considerable peptone was to be found in the blood of the mesenteric veins and more or less in

* Pflüger's Archiv, vol. xxviii, p. 123.

† Ueber Peptone und Ernahrung mit denselben. Pflüger's Archiv, vol. x, p. 536.

the liver, only the merest trace was to be found in the blood of the hepatic vein, indicating thereby a decomposition of peptone in its passage through the liver.

Maydl* claims that since the products of the decomposition of all forms of glycogen are the same, it follows that the glycogens themselves are all identical, and since it is extremely improbable that the various carbohydrates with their different chemical constitutions should give *one* glycogen, he argues that it all must come from one source, viz: albumin.

This is not the place, however, to discuss the relative merits of the dehydration and storage theories, it is enough simply to understand that the possible origin of liver sugar in proteid matter is one which would make clear many hitherto unexplained points. The great obstacle, has been to understand where and in what manner the liver sugar could be so formed. Seegen's views therefore are of great importance, and are, moreover, in no sense, wholly inconsistent with previous ideas, but the question at once suggests itself whether the analytical data on which they are founded are sufficient to warrant their adoption.

The determination of sugar in organic fluids is not without difficulty, and where slight variations in results may cause differences of half a per cent. or more, it becomes an extremely delicate matter to determine how far such results shall be trusted. Consequently, whatever may be said as to whether the formation of sugar in the manner indicated by Seegen is a natural or an artificial process, we need first of all to know positively whether the liver under any circumstances is able to form sugar or other carbohydrate matter from peptones. This all hinges on the accuracy of Seegen's results, obtained by warming portions of liver with peptones. If an increase of sugar and total carbohydrates is found in the presence of peptone, then we must conclude that the latter has at least some influence on the formation of the liver sugar. Recent experiments† have plainly shown that neutral peptone has a stimulating influence on the amylolytic action of ptyalin of saliva and diastase of malt; both of these ferments convert more starch into sugar in the presence of peptone than without and it is natural to suppose that the presence of peptone would similarly affect the amylolytic ferment which presumably acts upon glycogen. Seegen's results, however, appear to show that while sugar is increased

* Zeitschrift für physiol. Chem., vol. iii, p. 196. Ueber die Abstammung des Glykogens.

† Trans. Conn. Acad., vol. vi, p. 343, vol. vii, p. 44.

in the presence of peptone, glycogen remains nearly stationary, or if diminished, not at all in proportion to the increase in sugar. Boehm and Hoffmann, however, found the liver glycogen much less resistant and that its decrease was in proportion to the increase in sugar. Delprat,* too, came to similar conclusions and could obtain no proof whatever, of the correctness of the views advanced by Seegen and Kratschmer. We have, therefore, in view of the importance of the subject, undertaken a study of the question in the hopes of throwing some additional light upon the matter. In this, however, we have limited ourselves entirely to a study of the post-mortem formation of sugar and carbohydrates by the liver in the presence of peptones.

Methods employed.

The animals experimented with, mainly rabbits, were killed by severing the jugular vein, the blood being collected and defibrinated. The liver was quickly taken out, the gall bladder removed and the liver then converted into a fine pulp by chopping, since it is probable, as v. Wittich has suggested, that glycogen is unequally distributed through the liver. Two equal portions of the sampled and finely divided liver were accurately weighed out and placed in separate flasks; one, with a solution of peptone and a known volume of blood, the other with an amount of distilled water equal in volume to that of the two former. Both were then placed in a bath and warmed at 38–40° C. for the time of the experiment. A continuous current of air was made to pass through the blood solution in order to render it arterial. At the end of the experiment, the mixtures were poured into boiling water and extracted as long as a trace of glycogen could be detected in the fluids, by the iodine test. This usually took about two days, working on an average with 40 grams of liver. At the beginning of the extraction, the tissue was generally boiled with 400–500 c. c. of water for about fifteen minutes and then filtered through a funnel plugged with absorbent cotton. By repeating this operation four or five times, the greater portion of glycogen could be removed, but a complete extraction could be obtained only by long continued boiling with fresh quantities of water or long heating on the water-bath, the tissue being ground up occasionally in a suitable mortar. The various filtrates were evaporated on a water-bath and finally united and made up exactly to 500 c. c., after which the extracts were filtered through dry paper filters to

* Jahresbericht für Thierchemie, 1881, p. 321.

remove any traces of suspended matter which might have passed the cotton. Of these fluids, 200 c. c. of each were used for the determination of glycogen and sugar, and 200 c. c. also, for the determination of total carbohydrates.

Determination of glycogen and sugar.—The 200 c. c. of fluid for the determination of glycogen and sugar were evaporated to a small bulk and then, when cool, precipitated by a large volume of alcohol. After standing 24 hours the clear supernatant fluid was filtered from the precipitated glycogen and peptones. The alcoholic filtrate and washings, containing the sugar, were then evaporated, the residue dissolved in water and made up to 100 c. c., in an aliquot portion of which the sugar was determined gravimetrically, by Allihn's* improved method.

The precipitate of glycogen, with its frequent admixture of peptone, was dissolved in water, the solution made up to 200 c. c. and then sufficient 10 per cent. hydrochloric acid added to make the solution contain 2 per cent. HCl. The mixture was then heated in a closed flask at 100° C. for 17 hours in order to convert the glycogen into dextrose, after which the solution was neutralized, concentrated somewhat, again made up to 200 c. c. and in an aliquot portion of this fluid, dextrose was determined by Allihn's method, from which was calculated the amount and percentage of glycogen. Delprat† states that in attempting to determine glycogen by Brücke's method he found the results considerably higher than when the isolated glycogen was converted into sugar by boiling with acid and the glycogen calculated from the data obtained. In our own experiments, the frequent presence of peptone prevented entirely the use of Brücke's method. 12 hours heating at 100° C., however, with 2 per cent. hydrochloric acid was found in our case insufficient to completely convert the glycogen into dextrose, while 17 hours was found amply sufficient for complete conversion and at the same time allowed no decomposition of the sugar formed. This is well illustrated by the following experiments:

A. 0·7665 gram pure, dried glycogen dissolved in 100 c. c. of water, was heated at 100° C. for 12 hours with sufficient hydrochloric acid to make the entire fluid contain exactly 2 per cent. The solution was neutralized, care being taken that the reaction did not become alkaline, then concentrated and finally made up to 50 c. c.

14 c. c. gave 0·4215 gram Cu=0·2251 gram dextrose=0·2025 gram glycogen.
14 " " 0·4233 " Cu=0·2263 " " =0·2036 "

* Zeitschrift für analytische Chemie, xxii, p. 448.

† Jahresbericht für Thierchemie, 1881, p. 322.

The 14 c. c. should have contained 0·2414 gram dextrose, the equivalent of 0·2146 gram of glycogen.

B. 0·6190 gram glycogen dissolved in 100 c. c. of water was heated at 100° C. for 17 hours in the presence of 2 per cent. of hydrochloric acid. Solution was then neutralized, evaporated and made up to 50 c.c.

18 c.c. gave 0·4585 gram Cu=0·2470 gram dextrose=0·2223 gram glycogen.
18 " " 0·4555 " Cu=0·2454 " =0·2208 "

The 18 c. c. should have contained 0·2475 gram dextrose, equal to 0·2228 gram of glycogen. Hence, it is seen that 17 hours heating at 100° C. is needed for a complete conversion of glycogen into dextrose, which was the time invariably employed in the after experiments.

Influence of peptone on the conversion of glycogen into sugar by 2 per cent. HCl at 100° *C.*—The question naturally suggested itself in this connection whether the presence of peptone would interfere in any way with the complete conversion of glycogen into dextrose or whether the peptones by this long heating at 100° C. with the acid, would undergo any change by which reducing bodies might be formed and thus endanger the accuracy of the results. The latter point was tested by heating 2 grams of peptones in 100 c. c. of water containing 2 per cent. of hydrochloric acid for 17 hours at 100° C., at the end of which time no reduction at all could be obtained with Fehling's solution.

The first point was tested by the following experiment:

0·9290 gram of pure glycogen was dissolved in 100 c. c. of water, then 2 grams of peptone were added and sufficient acid for the solution to contain exactly 2 per cent. HCl, after which the mixture was heated at 100° C. for 17 hours. The solution was then neutralized, brought to a volume of 100 c. c. and the sugar determined.

10 c. c. gave 0·1985 gram Cu=0·1017 gram dextrose=0·0915 gram glycogen.
10 " " 0·2025 " Cu=0·1039 " =0·0934 "

whereas in the 10 c. c. then should be present, according to calculation 0·1032 gram dextrose, the equivalent of 0·0929 gram of glycogen. Consequently the presence of peptone does not interfere with the accurate determination of glycogen by this method.

Influence of the presence of peptone on the determination of sugar by Allihn's method.—Seegen* finds that the volumetric determination of sugar with Fehling's solution is not materially affected by the presence of peptone. By repeated experiments we have convinced ourselves, that in the use of the gravimetric method, the

* Pflüger's Archiv, vol. xxviii, p. 115.

presence of peptone may, unless certain precautions are taken, interfere slightly with exact determinations. With the Allihn method, variations of 2–5 milligrams in the amount of reduced copper are liable to occur if care is not taken in regulating the length of time the alkaline copper solution is heated after addition of the sugar solution. Under ordinary circumstances results most nearly in accord with theory are obtained by adding the sugar solution, as recommended by Allihn, to the previously heated Fehling's solution and then heating further until bubbles just begin to break upon the surface of the liquid. If heated longer, even only half a minute, a slight increase in the amount of reduced copper will generally be observed. Now whenever peptone is present to any extent in the sugar solution, we have found by experience that complete reduction does not take place quite so rapidly; the loss is not great, sometimes but a milligram or so, still the difference is appreciable. This, however, can be avoided by simply allowing the standard copper solution to boil for about 45 seconds after the addition of the sugar solution. Under such conditions, repeated trials have shown us, that the presence of peptone does not offer the slightest obstacle to accurate determinations of dextrose. Whenever, therefore, in the following experiments the solution to be tested contained peptone, the above rule has been invariably followed.

Determination of total carbohydrates.—For this purpose 200 c. c. of the liver extract were heated in a closed flask at 100° C. with sufficient 10 per cent. hydrochloric acid to ensure a content of 2 per cent HCl, for 17 hours. The solution was then nearly neutralized, care being taken that the fluid did not become alkaline, concentrated and finally brought to a volume of 200 c. c., in an aliquot portion of which the total carbohydrates in the form of dextrose were determined in the usual manner. Seegen* states that in the determination of total carbohydrates, the fluid, after heating with acid, always became very dark, which occasionally interfered somewhat with the determination of sugar. Delprat,† however, states that in his experiments the solution, under like conditions, became brownish yellow and generally deposited a flocculent brownish black precipitate of organic matter. Moreover, in some cases, particularly with the livers of dogs, cats and calves, the cuprous oxide, in determining total carbohydrates, would remain dissolved to a great extent, thus interfering with the accuracy of the volumetric determination, some-

* Pflüger's Archiv, vol. xxviii, p. 121.

† Jahresbericht für Thierchemie, 1881, p. 323–324.

times to the extent of even 1–2 c. c. of the sugar solution. In our experiments the acid solution was usually yellow or yellowish brown, and invariably at the end of the 17 hours contained the flocculent precipitate described by Delprat. By nearly neutralizing the solution, the amount of this precipitate was considerably increased and on then filtering the fluid, after having made it up to a volume of 200 c. c., considerable organic matter was removed. This, we found, had a decided influence on the accuracy of the determination, since alkaline solutions of this neutralization precipitate appeared to decidedly retard separation of the cuprous oxide. By paying attention to this point, we had no difficulty in obtaining fairly concordant results, by the use of the Allihn gravimetric method.

Experiment I.

A large sized rabbit was killed, the blood collected and defibrinated, the liver quickly removed and finely chopped. Two portions of 40 grams each were weighed out and treated as follows:

A.	*B.*
40 grams liver.	40 grams liver.
50 c. c. of a 10 % solution of peptone.	95 c. c. of water.
25 c. c. of blood.	
20 c. c. of water.	

These were placed in flasks, and warmed at 40° C. for two hours. The liver was in contact with the peptone 40 minutes after the death of the animal. A continuous current of air was kept passing through *A*. Following are the analytical results:

Glycogen; total volume of the resultant sugar solution 200 c. c.
Sugar; total volume of the solution 100 c. c.
Total carbohydrates; volume of the resultant solution 200 c. c.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amt.*	Per cent.
		Glycogen A.			
25 c. c.	0·2345 gram.	0·1209 gram.	0·1088 gram.	0·8704 gram.	5·44
25	0·2360	0·1217	0·1095	0·8760	5·47
		Glycogen B.			
25 c. c.	0·2675 gram.	0·1386 gram.	0·1247 gram.	0·9976 gram.	6·23
25	0·2659	0·1377	0·1239	0·9912	6·19
		Sugar A.			
25 c. c.	0·2260 gram.	0·1164 gram.	-----	0·4656 gram.	2·91
25	0·2255	0·1163	-----	0·4652	2·90

* Total amount of glycogen, dextrose or carbohydrates calculated as dextrose, contained in the above volume (100 or 200 c. c.) and representing, therefore, the amount contained in two-fifths of the 40 grams of liver.

Sugar B.

Volume used.	Weight Cu.	Equivalent in dextrose.	Total amt.	Per cent.
25 c. c.	0·2135 gram.	0·1097 gram.	0·4388 gram.	2·74

Total Carbohydrates A.

12·5 c. c.	0·2160 gram.	0·1111 gram.	1·7776 grams.	11·10
20	0·3367	0·1768	1·7680	11·05

Total Carbohydrates B.

25 c. c.	0·4030 gram.	0·2146 gram.	1·7168 grams.	10·73
25	0·4042	0·2153	1·7224	10·76

The following table shows the average percentage results:

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood (A),	5·46 %	2·91 %	11·08 %
40	Without peptones and blood (B),	6·21	2·74	10·75
		−0·75	+0·17	+0·33

From this it is seen that while in the presence of peptone and blood there is a slight increase of both total carbohydrates and sugar, there is also a more than corresponding decrease in the percentage of glycogen.

Experiment II.

Liver of a rabbit, removed directly after death and treated in the same manner as in Experiment I.

A.	*B.*
40 grams sampled liver.	40 grams sampled liver.
50 c. c. of a 10 per cent. peptone solution.	145 c. c. of water.
25 grams blood.	
70 c. c. of water.	

Warmed 2 hours at 40° C., with a current of air passing through *A*.

Glycogen A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·3170 gram.	0·1659 gram.	0·1493 gram.	1·1944 grams.	7·46

Glycogen B.

25 c. c.	0·3420 gram.	0·1798 gram.	0·1618 gram.	1·2944 grams.	8·09

Sugar A.

25 c. c.	0·2520 gram.	0·1303 gram.	-----	0·5212 gram.	3·26

Sugar B.

10 c. c.	0·0865 gram.	0·0441 gram.	-----	0·4410 gram.	2·75

Total carbohydrates A.

10 c. c.	0·2205 gram.	0·1134 gram.	-----	2·2680 grams.	14·15

Total carbohydrates B.

10 c. c.	0·2110 gram.	0·1084 gram.	-----	2·1680 grams.	13·55

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood (A),	7·46 %	3·26 %	14·15 %
40	Without peptones and blood (B),	8·09	2·75	13·55
		−0·63	+0·51	+0·60

Experiment III.

A small rabbit, treated in the same manner as the preceding:

A.	*B.*
25 grams sampled liver.	25 grams sampled liver.
50 c. c. of a 10 per cent. peptone solution.	125 c. c. of water.
50 grams of blood.	
25 c. c. of water.	

Warmed for 2 hours at 40° C., with a constant current of air passing through *A*.

Glycogen A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·0435 gram.	0·0226 gram.	0·0203 gram.	0·1624 gram.	1·62
25	0·0455	0·0236	0·0212	0·1696	1·69

Glycogen B.

25 c. c.	0·0425 gram.	0·0221 gram.	0·0198 gram.	0·1584 gram.	1·58
25	0·0405	0·0211	0·0189	0·1512	1·51

*Sugar B.**

25 c. c.	0·1413 gram.	0·0719 gram.	-----	0·2876 gram.	2·87
25	0·1400	0·0713	-----	0·2852	2·85

Total carbohydrates A.

25 c. c.	0·1560 gram.	0·0796 gram.	-----	0·6368 gram.	6·36
25	0·1585	0·0809	-----	0·6472	6·47

Total carbohydrates B.

25 c. c.	0·1445 gram.	0·0736 gram.	-----	0·5888 gram.	5·88
25	0·1427	0·0726	-----	0·5808	5·80

Following are the average percentage results:

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
25 grams.	With peptones and blood (A),	1·65 %	----	6·42 %
25	Without peptones and blood (B),	1·54	2·86 %	5·84
		+0·11		+0·58

In this experiment there is the same slight increase of total carbohydrates in the presence of peptone noticed in the two preceding experiments. The increase, however, is not great, and it suggests at once the question, whether the differences, although constant, are

* Sugar *A* was lost.

beyond the ordinary limits of error. This question we have endeavored to answer in the next experiment.

Experiment IV.

A rabbit's liver removed from the body immediately after death, was prepared in the usual manner. Two mixtures, exactly alike, were then made as follows:

A.	*B.*
25 grams of liver.	25 grams of liver.
100 c. c. of water.	100 c. c. of water.

These were in the bath 23 minutes after the death of the animal and were warmed at 40° C. for 2 hours. The two portions were then extracted and analyzed as in the preceding experiments; the object being to see how great a variation would be obtained by this like treatment of the two portions of sampled liver. Following are the results:

Glycogen A.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·1575 gram.	0·0802 gram.	0·0721 gram.	0·5768 gram.	5·76

Glycogen B.

25 c. c.	0·1585 gram.	0·0809 gram.	0·0728 gram.	0·5824 gram.	5·82

Sugar A.

10 c. c.	0·0433 gram.	0·0225 gram.	-----	0·2250 gram.	2·25

Sugar B.

10 c. c.	0·0430 gram.	0·0224 gram.	-----	0·2240 gram.	2·24

Total carbohydrates A.

25 c. c.	0·2340 gram.	0·1207 gram.	-----	0·9656 gram.	9·65

Total carbohydrates B.

25 c. c.	0·2335 gram.	0·1203 gram.	-----	0·9624 gram.	9·62

Percentage results.

	Glycogen.	Sugar.	Total carbohydrates.
A.	5·76 per cent.	2·25 per cent.	9·65 per cent.
B.	5·82	2·24	9·62
	−0·06	+0·01	+0·03

These results plainly show that when the conditions of the experiment are exactly the same, the average variation in results will be considerably less than 0·1 per cent. Consequently variations greater than this must have their origin in something other than the ordinary errors of analysis. Hence, in the three preceding experiments we have to account for an average increase of about 0·5 per cent. in

total carbohydrates in those cases where peptone and blood are both present.

A comparison of Seegen's results* show that while an aqueous solution of peptone alone in contact with fresh liver increases somewhat the percentage of both sugar and total carbohydrates, the addition of blood, kept arterial by the passage of a current of air through the fluid, appears to still further increase the percentage of sugar and carbohydrates. Seegen, moreover, shows by a blank experiment, that blood alone in contact with the liver has no more influence on the formation of carbohydrates than distilled water.

Experiment V.

This experiment was tried mainly to see what influence peptones by themselves in the absence of blood, would have on the formation of sugar and total carbohydrates. Two portions of sampled liver from a large rabbit were treated as follows:

A.	*B.*
50 grams liver.	50 grams liver.
50 c. c. of water containing 2 grams of peptones.	50 c. c. of water.

The solution of peptone was poured over the liver just 45 minutes after the death of the animal. The mixtures were placed in a bath at 40° C. for 3 hours, after which they were allowed to stand at the temperature of the room for 21 hours. They were then extracted and analyzed in the usual manner, with the following results:

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c. c.	0·1900 gram.	0·0973 gram.	0·0875 gram.	0·7000 gram.	3·50
25	0·1915	0·0980	0·0882	0·7056	3·52
		Glycogen B.			
25 c. c.	0·1785 gram.	0·0913 gram.	0·0821 gram.	0·6568 gram.	3·28
		Sugar A.			
25 c. c.	0·4085 gram.	0·2177 gram.	-----	0·8708 gram.	4·35
10	0·1735	0·0887	-----	0·8870	4·43
		Sugar B.			
10 c. c.	0·1745 gram.	0·0892 gram.	-----	0·8920 gram.	4·46
		Total carbohydrates A.			
10 c. c.	0·1985 gram.	0·1017 gram.	-----	2·0340 grams.	10·17
		Total carbohydrates B.			
10 c. c.	0·1830 gram.	0·0937 gram.	-----	1·8740 grams.	9·37

* Pflüger's Archiv, vol. xxv, p. 172; ibid, vol. xxviii, p. 125.

Average percentage results.

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams	With peptones (A),	3·51 per cent.	4·39 per cent.	10·17 per cent.
50	Without peptones (B),	3·28	4·46	9·37
		+0·23	−0·07	+0·80

Experiment VI.

This experiment is practically a repetition of the preceding one, excepting that the temperature throughout the experiment was about 18–20° C. and the length of time 24 hours. This latter point we deem of considerable importance, for as the results show, the same increase in total carbohydrates in the presence of peptone is apparent here, after 24 hours treatment and also in the preceding experiment after 21 hours treatment, as has been observed at the end of 2 to 3 hours in the presence of peptone and blood. For Seegen lays considerable stress upon the fact that in many animals the newly-formed carbohydrates and sugar, supposed to have their origin in the peptones, are after a time decomposed, so that at the end, say of 24 hours, the content of sugar and total carbohydrates fall back to their original amount, that is, the amount found in the control. Seegen further claims that this point speaks strongly in favor of the action of the liver, as such, on the conversion of peptone, and rabbits' liver according to his experiments is no exception to the rule.* The experiment was as follows:

A.	*B.*
40 grams fresh liver (rabbit).	40 grams fresh liver.
55 c. c. of water containing 2 grams of peptone.	55 c. c. of water.

These two mixtures stood at the temperature of the room for 24 hours, when they were extracted and analyzed with the following results:

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c. c.	0·2767 gram.	0·1436 gram.	0·1292 gram.	1·0336 grams.	6·46
		Glycogen B.			
25 c. c.	0·2515 gram.	0·1299 gram.	0·1168 gram.	0·9344 gram.	5·84
		Sugar A.			
25 c. c.	0·2745 gram.	0·1424 gram.	-----	0·5696 gram.	†3·56
25	0·2655	0·1375	-----	0·5500	3·43
		Sugar B.			
25 c. c.	0·3210 gram.	0·1681 gram.	-----	0·6724 gram.	4·20
25	0·3260	0·1709	-----	0·6836	4·27
		Total carbohydrates A.			
10 c. c.	0·2100 gram.	0·1079 gram.	-----	2·1580 grams.	13·48
		Total carbohydrates B.			
10 c. c.	0·2013 gram.	0·1032 gram.	-----	2·0640 grams.	12·90

* Pflüger's Archiv, vol. xxv, p. 175.

† In this determination, difficulty was experienced in obtaining a good reduction.

Average percentage results.

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams	With peptones (A),	6·46 per cent.	3·49 per cent.	13·48 per cent.
40	Without peptones (B),	5·84	4·23	12·90
		+0·62	−0·74	+0·58

Both of these experiments tend to show that the presence of blood has no especial influence on the percentage of total carbohydrates; fully as great an increase is to be noticed in the presence of peptone without blood, as when the latter is present. Evidently then, if the increase in total carbohydrates noticed in all of our experiments is really due to the post-mortem formation of carbohydrate matter from peptone it is quite certain that blood is not at all essential to the reaction, at least in the livers of rabbits.

It is to be noticed, moreover, in the two last experiments that, in the absence of blood, the sugar is not increased in amount in the presence of peptone. On the contrary, the presence of peptone under such conditions appears to diminish the formation of sugar, glycogen being correspondingly increased. In all of the experiments with rabbits, it is apparent from the results, that any increase of sugar in the presence of peptone is in every instance counterbalanced by a corresponding decrease in glycogen. In the last two experiments, the same relationship between the amount of glycogen and sugar is to be noticed, only here the greatest percentage of sugar is to be found in that portion of the liver which was treated without peptones. This would suggest that blood either facilitates in some manner the action of such amylolytic ferment as is present in the liver, or else that it introduces an additional ferment which causes increased amylolytic action. Blood certainly does not contain any substance convertible into sugar by the action of boiling acids, since the increase in total carbohydrates is no greater in the presence of blood than in the presence of peptone alone.

We have therefore tried the following experiment in order to ascertain whether blood by itself, in the absence of peptone, has any influence whatever on the formation of sugar.

Experiment VII.

A.	*B.*
50 grams of sampled liver (rabbit).	50 grams of sampled liver.
27 grams of blood.	92 c. c. of water.
65 c. c. of water.	

The blood from the same rabbit was poured over the liver 35 minutes after the death of the animal and the two flasks containing the

mixtures were then placed at a temperature of 40° C. for 2 hours. A constant current of air was kept passing through the blood during this time. Following are the results obtained:

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c. c.	0·1270 gram.	0·0647 gram.	0·0582 gram.	0·4656 gram.	2·32
		Glycogen B.			
25 c. c.	0·1947 gram.	0·0997 gram.	0·0897 gram.	0·7176 gram.	3·58
		Sugar A.			
10 c. c.	0·1635 gram.	0·0845 gram.	-----	0·8450 gram.	4·22
		Sugar B.			
10 c. c.	0·1420 gram.	0·0723 gram.	-----	0·7230 gram.	3·61
10	0·1415	0·0720	-----	0·7200	3·60
		Total carbohydrates A.			
20 c. c.	0·3800 gram.	0·2014 gram.	-----	2·0140	10·07
		Total carbohydrates B.			
20 c. c.	0·3785 gram.	0·2005 gram.	-----	2·0050	10·02

Average percentage results.

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With blood (A),	2·32 per cent.	4·21 per cent.	10·07 per cent
50	Without blood (B),	3·58	3·61	10·02
		−1·26	+0·60	+0·05

Total carbohydrates are not at all affected by the presence of blood, but the percentage of sugar is considerably increased, and in accord with the increase of sugar, is to be noticed a decided decrease in the percentage of glycogen. Evidently then the percentage of sugar in the rabbit's liver is increased in the presence of blood, which increase is due wholly to a more vigorous decomposition of glycogen. Moreover, whenever increase of sugar has been observed in our experiments a corresponding decrease in glycogen has as a rule, also been seen. In this respect, therefore, our results agree with those of Boehm and Hoffmann, as also with those of Delprat. Seegen, however, states in a later communication,* that in rabbits, glycogen is more rapidly changed than in the case of dogs, or in other words that the liver-glycogen of rabbits is less resistant to the action of ferments.

In all of the preceding experiments with the livers of rabbits, it is to be noticed that the sum of glycogen, calculated as dextrose, and

* Pflüger's Archiv, vol. xxiv, p. 467.

the sugar, is not at all equal to the figures representing total carbohydrates, being in every instance considerably less than the latter. Thus in Experiment V, the amount of total carbohydrates is in *A* (with peptones) 10·17 per cent., while the sum of sugar and glycogen calculated as dextrose is but 8·29 per cent.; a deficit of 1·88 per cent. In *B*, likewise, where peptones are not present, there is a similar deficit, amounting in this case to 1·27 per cent. It is not to be supposed that such a deficiency could in any manner arise from errors of analysis and the most natural supposition is that the sugar, determined and calculated as dextrose, might be of lower reducing power; or in other words that it might consist of maltose instead of dextrose, or rather, of a mixture of maltose and dextrose or of a soluble dextrin. O. Nasse* has stated that the dead liver contains dextrose, or a sugar whose reducing power is not increased by heating with dilute sulphuric acid. Seegen and Kratschmer† also state that the dead liver contains dextrose, and further, that the liver sugar is *exclusively* dextrose. This opinion is based mainly upon the fact that the fluid obtained from a calf's liver by pressure, yielded by dialysis and subsequent treatment with alcohol, a saccharine body, which on the addition of an alcoholic solution of potash was converted into the known *dextrose-potash* compound. Musculus and V. Mering,‡ however, claim that in addition to dextrose, the dead liver also contains maltose. This sugar they detected twice; once in the dead liver of a dog, 1 hour after death, and again, also in a dog's liver, 5 hours after death. In both cases dextrose was likewise present. Dextrin they were not able to detect with certainty, but they consider that the liver ferment also forms this body, intermediate between glycogen and the sugars. E. Külz§ has also prepared from the dead livers of dogs pure dextrose, but he does not conclude definitely as to the presence of dextrin and maltose. It is to be seen, therefore, that while there is unanimity of opinion regarding the presence of dextrose, there is less certainty regarding the presence of the lower reducing sugar, maltose.

We have therefore, carefully examined the nature of the sugar remaining in the alcoholic filtrate after the precipitation of glycogen, and we find, in almost every instance, that the saccharine body there

* Bemerkungen zur Physiologie der Kohlehydrate. Pflüger's Archiv, vol. xiv, p. 473.

† Die Natur des Leberzucker. Pflüger's Archiv, vol. xxii, p. 214.

‡ Ueber die Umwandlung von Stärke und Glycogen durch Diastas, Speichel, Pancreas und Leberferment. Zeitschrift für physiologische Chemie, vol. ii, p. 417.

§ Ueber die Natur des Zuckers in der todtenstarren Leber. Pflüger's Archiv, vol. xxiv, p. 52.

present, has its reducing power considerably increased by heating for a short time with 2 per cent. sulphuric acid. Moreover, the sum of glycogen calculated as dextrose, and the liver sugar converted wholly into dextrose by boiling with dilute acid, exactly equals the total carbohydrates in those cases where peptones are not present. Thus in Experiment V, *A* and *B*, the final sugar solution amounted in each instance to 100 c. c.; 50 c. c. of these solutions were mixed with sufficient 10 per cent. sulphuric acid to insure a content of 2 per cent., after which the two solutions were boiled for two hours, evaporation being prevented by an inverted Liebig's condenser. On cooling, the solutions were made nearly neutral, concentrated somewhat and finally brought back to a volume of exactly 50 c. c. Following are the analytical results both before and after boiling with the dilute acid.

A. With peptones.

	Volume used.	Weight Cu.	Equivalent in dextrose.	Total amount.	Per cent.
Before boiling,	10 c. c.	0·1735 gram.	0·0887 gram.	0·8870 gram.	4·43
After boiling,	10	0·2270	0·1169	1·1690	5·84

B. Without peptones.

Before boiling.	10 c. c.	0·1745 gram.	0·0892 gram.	0·8920 gram.	4·46
After boiling,	10	0·2234	0·1150	1·1500	5·75

Hence, it is evident that the liver sugar, in this instance at least, is not made up entirely of dextrose. Neither is it wholly maltose, for if such were the case, the reducing power before and after boiling with acid, would be in the proportion of 66 : 100, whereas in the above experiment the ratio in *A* is 76·4 : 100 and in *B* 78·1 : 100. Still, it would appear that the lower reducing body is present in the largest quantity.

It was not our purpose to study particularly the nature of the liver sugar, so we have not sought for positive proof of the character of this lower reducing body. That it is not dextrin, or glycogen left unprecipitated by the alcohol, is evident from the fact that the addition of a little pure yeast to the normal sugar solution sets up a fermentation by which the sugars are not only completely decomposed but no amylaceous bodies whatever remain in the fluid; at least none which will yield reducing bodies on boiling with dilute sulphuric acid. Consequently it would appear that the lower reducing body is in all probability maltose. We do not intend to say, however, that dextrin is never present in the liver; on the contrary, we are inclined to agree with Musculus and v. Mering that dextrin is doubtless formed as an antecedent to maltose, but in the two or

three fermentation experiments that we have tried, the sugar solution has never contained dextrin. It would of course be more natural in seeking for dextrin to look in the glycogen precipitate. Finally, it is interesting to notice in connection with this same experiment, that in *B* the sum of glycogen calculated as dextrose and the sugar after boiling with sulphuric acid equals the total carbohydrates found; while in *A* where peptones are present, the total carbohydrates more than equal the sum of glycogen and sugar, as is shown by the following figures from Experiment V.

	Sugar not boiled with acid.		Sugar boiled with acid.	
	A.	*B.*	*A.*	*B.*
Glycogen calculated as dextrose,	3·90 p. c.	3·64 p. c.	3·90 p. c.	3·64 p. c.
Sugar calculated as dextrose,	4·43	4·46	5·84	5·75
	8·33	8·10	9·74	9·39

Total carbohydrates found in *A* 10·17 per cent.
" " " *B* 9·37

This shows an apparent increase in total carbohydrates of 0·43 per cent. (in A) under the influence of the peptones; this being the difference between the total carbohydrates found, and the sum of glycogen calculated as dextrose and the sugar after boiling with dilute acid. In *B* on the other hand, the total carbohydrates found and the sum of glycogen and sugar differ but 0·02 per cent.

Experiment VIII.

This experiment was tried mainly to verify the preceding statements regarding the liver sugar.

40 grams of liver from a rabbit were finely divided and placed in a flask with 50 c. c. of water. The mixture was then warmed at 38–40° C. for 2 hours, when it was boiled and extracted in the usual manner. The liver was placed in the warm bath just 25 minutes after the death of the rabbit, consequently 2½ hours intervened between the death of the animal and the stopping of ferment action. Following are the results of the analysis:

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen.			
25 c. c.	0·2990 gram.	0·1560 gram.	0·1404 gram.	1·1232 grams.	7·02
		Sugar.			
10 c. c.	0·0915 gram.	0·0466 gram.	-----	0·4660 gram.	2·91
		Sugar after boiling with dilute H_2SO_4.			
10 c. c.	0·1145 gram.	0·0582 gram.	-----	0·5820 gram.	3·63
		Total carbohydrates.			
10 c. c.	0·1790 gram.	0·0916 gram.	-----	1·8320 grams.	11·45

Glycogen as dextrose,	7·80 per cent.		7·80 per cent.
Sugar,	2·91	After boiling with H_2SO_4,	3·63
	10·71		11·43

It is evident that here, as in the preceding experiment, the sum of glycogen calculated as dextrose and the sugar equals the total carbohydrates actually found, only when the sugar has been boiled with dilute sulphuric acid, in which case the agreement is, as before, almost exact. Plainly then, dextrose is not the only sugar formed in the liver, and if, as seems probable from our experiments, the sugar has its origin in the hepatic glycogen, it would be quite in accord with analogy to expect the presence of both maltose and dextrose.

The relative reducing power, before and after boiling with acid, is much the same as in the preceding experiments, viz: 79·9 : 100, and indicates the presence of considerable of the body with lower reducing power. This portion of the experiment was duplicated with another 40 grams of liver from the same rabbit under exactly the same conditions as to time and temperature, with results almost identical with those obtained from the preceding portion, thus testifying to the accuracy of the methods.

Sugar solution before boiling with dilute acid.

10 c. c. gave 0·0865 gram Cu=0·0444 gram dextrose=2·78 per cent.

Sugar solution after boiling with dilute acid.

10 c. c. gave 0·1080 gram Cu=0·0550 gram dextrose=3·44 per cent.

The relative reducing power before and after boiling with dilute sulphuric acid is in this case 80·0 : 100.

Experiments were now tried, to ascertain the influence of peptone on the formation of carbohydrates in the livers of other animals.

Experiment IX.

A large cat in full digestion, fed mainly on proteid matter, was chloroformed, blood collected from the jugular vein and the liver at once removed. Two mixtures were then prepared as follows:

A.	*B.*
50 grams sampled liver,	50 grams sampled liver.
20 grams blood,	100 c. c. of water.
50 c. c. of a 10 per cent. solution of peptone.	
40 c. c. of water.	

These were placed in a bath at 38–40° C. 50 minutes after the death of the animal, and were kept there for 2½ hours, with a constant current of air passing through *A* in order to render the blood

arterial. At the end of this time further action was prevented by boiling the mixtures, after which they were extracted in the usual manner. Neither of the two extracts contained enough glycogen for estimation. Following are the other results :

Sugar A.

Volume used.	Weight of Cu.	Equivalent in dextrose.	Total amount.	Per cent.
25 c. c.	0·1710 gram.	0·0874 gram.	0·3496 gram.	1·74
	After boiling with dilute H_2SO_4.			
25 c. c.	0·1730 gram.	0·0885 gram.	0·3540 gram.	1·77
	Sugar B.			
25 c. c.	0·1638 gram.	0·0837 gram.	0·3348 gram.	1·67
	After boiling with dilute H_2SO_4.			
25 c. c.	0·1750 gram.	0·0895 gram.	0·3580 gram.	1·79
	Total carbohydrates A.			
25 c. c.	0·1048 gram.	0·0533 gram.	0·4264 gram.	2·13
	Total carbohydrates B.			
25 c. c.	0·0930 gram.	0·0474 gram.	0·3792 gram.	1·89

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar. Before boiling.	Sugar. After boiling.	Total carbohydrates.
50 grams.	With peptones and blood (A),	0	1·74 %	1·77 %	2·13 %
50	Without peptones and blood (B),	0	1·67	1·79	1·89
			+0·07	−0 02	+0·24

In the presence of peptone, there is to be noticed a slight increase in total carbohydrates, which increase receives confirmation from the fact that in *A*, the total carbohydrates exceed the sugar by 0·36 per cent., while in *B* the difference after boiling the sugar with dilute acid is but 0·1 per cent., probably within the limits of error. It is to be noticed, moreover, that in the presence of peptone, the sugar formed is wholly dextrose, its reducing power not being materially changed by boiling with dilute acid, whereas in *B* there is a slight increase in reducing power on boiling with acid. If the difference is sufficient to warrant any explanation it might be suggested that peptone by its presence* stimulates the ferment which presumably converts maltose into dextrose and thus in *A* complete conversion was effected sooner than in *B*.

Experiment X.

This experiment was like the preceding, with the exception that the cat employed was not in full digestion. The stomach was nearly

* Compare Chittenden and Smith, Trans. Conn. Acad., vol. vi, p. 343.

empty and the small intestines likewise. The sampled liver was in the bath at 40° C., 25 minutes after the death of the animal.

A.	*B.*
40 grams of liver.	40 grams of liver.
25 grams of blood.	135 c. c. of water.
100 c. c. of a 2 per cent. solution of peptone.	
10 c. c. of water.	

These mixtures were warmed at 40° C. for 2 hours, with a current of air passing through *A*, after which they were extracted and treated in the usual manner. Glycogen was not found in sufficient quantity for determination in either *A* or *B*. An accident happened to the sugar solutions, so that the absolute amount of sugar could not be estimated, but the relative reducing power of the sugar solution, before and after boiling with dilute sulphuric acid, was accurately determined.

Volume used.	Weight of Cu.	Equivalent in dextrose.	Total amount.	Per cent.
		Total carbohydrates A.		
25 c. c.	0·1070 gram.	0·0545 gram.	0·4360 gram.	2·72
		Total carbohydrates B.		
25 c. c.	0·0865 gram.	0·0441 gram.	0·3528 gram.	2·20
		Sugar A.		
25 c. c.	0·1045 gram.	0·0529 gram.	Before boiling with dilute acid.	
25	0·1479	0·0753	After boiling with acid.	
		Sugar B.		
25 c. c.	0·1453 gram.	0·0740 gram.	Before boiling with dilute acid.	
25	0·1925	0·0986	After boiling with acid.	

In this case, the total carbohydrates show an increase of 0·52 per cent. in the presence of peptone. The sugars, unlike the result in the preceding experiment, show a very great increase in reducing power, on being boiled with dilute sulphuric acid. In *A*, the relative reducing power before and after boiling with the acid, is 70·6 : 100 and in *B* 75·4 : 100. Hence in this experiment it would appear that the lower reducing body, presumably maltose, is present in great excess.

Experiment XI.

In this experiment, a liver was taken from a freshly killed lamb, sampled in the usual manner and then warmed at 40° C., with and without peptone, for 4 hours. The liver was in the bath and mixed with the peptone solution just one hour and 20 minutes after the death of the animal. In this experiment no blood was used.

A.	*B.*
50 grams of liver.	50 grams of liver.
50 c. c. of a 4 per cent. solution of peptone.	50 c. c. of water.

Glycogen could not be detected in sufficient quantity for estimation. Following are the other results:

Sugar A.

Volume used.	Weight of Cu.	Equivalent in dextrose.	Total amount.	Per cent.
25 c. c.	0·2440 gram.	0·1260 gram.	0·5040 gram.	2·52
		After boiling with dilute H_2SO_4.		
25 c. c.	0·2495 gram.	0·1289 gram.	0·5156 gram.	2·57
		Sugar B.		
25 c. c.	0·2430 gram.	0·1255 gram.	0·5020 gram.	2·51
		After boiling with dilute H_2SO_4.		
25 c. c.	0·2445 gram.	0·1262 gram.	0·5048 gram.	2·52
		Total carbohydrates A.		
25 c. c.	0·1185 gram.	0·0603 gram.	0·4824 gram.	2·41
		Total carbohydrates B.		
25 c. c.	0·1210 gram.	0·0616 gram.	0·4928 gram.	2·46

Here there is neither increase in total carbohydrates in the presence of peptone, nor is there any change in the reducing power of the sugar solutions after boiling with dilute acid. Total carbohydrates, moreover, fall a trifle below the percentage of sugar, although the difference is within the limits of error.

Experiment XII.

With a sheep's liver, 24 hours after death.

A.	*B.*
50 grams of sampled liver.	50 grams of sampled liver.
100 c. c. of a 2 per cent. solution of peptone.	100 c. c. of water.

These were warmed at 40° C. for 1½ hours, then extracted and tested. No glycogen was found.

Sugar A.

Volume used.	Weight of Cu.	Equivalent in dextrose.	Total amount.	Per cent.
25 c. c.	0·1085 gram.	0·0552 gram.	0·2208 gram.	1·10
		Sugar B.		
25 c. c.	0·0825 gram.	0·0420 gram.	0·1680 gram.	0·84
		Total carbohydrates A.		
25 c. c.	0·0750 gram.	0·0383 gram.	0·3064 gram.	1·53
		Total carbohydrates B.		
25 c. c.	0·0465 gram.	0·0241 gram.	0·1928 gram.	0·96

Amount of liver taken.	Method of treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones (A),	0	1·10 per cent.	1·53 per cent.
50	Without peptones (B),	0	0·84	0·96
			+0·26	+0·57

Increase in both sugar and total carbohydrates is to be noticed in *A*. The increase in total carbohydrates, moreover, is twice as great as the increase in sugar.

Experiment XIII.

With a calf's liver, from a freshly killed animal, obtained at the slaughter house.

A.	*B.*	*C.*
50 grams of liver.	50 grams of liver.	50 grams of liver.
100 c. c. of water.	100 c. c. of water.	100 c. c. of a 2 per cent. solution of peptone.

A was warmed at 40° C. for 1 hour and 20 minutes, after which it was extracted and analyzed; *B* and *C* on the other hand, were heated for 1 hour and 30 minutes at the same temperature, after which they were allowed to stand for 16 hours at about 18–20° C. before being extracted. The special object in view was to ascertain the influence of time on the disappearance of glycogen.

Following are the results obtained:

Volume used.	Weight of Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
		Glycogen A.			
25 c. c.	0·0230 gram.	0·0125 gram.	0·0112 gram.	0·0896 gram.	0·44
		Glycogen B.			
25 c. c.	0·0105 gram.	0·0063 gram.	0·0056 gram.	0·0448 gram.	0·22
		Glycogen C.			
25 c. c.	a mere trace only.				
		Sugar A.			
25 c. c.	0·1900 gram.	0·0973 gram.	-----	0·3892 gram.	1·94
		After boiling with dilute H_2SO_4.			
25 c. c.	0·2100 gram.	0·1079 gram.	-----	0·4316 gram.	2·15
		Sugar B.			
25 c. c.	0·2445 gram.	0·1262 gram.	-----	0·5048 gram.	2·52
		After boiling with dilute H_2SO_4.			
25 c. c.	0·2535 gram.	0·1310 gram.	-----	0·5240 gram.	2·62
		Sugar C.			
25 c. c.	0·2315 gram.	0·1198 gram.	-----	0·4792 gram.	2·39
		After boiling with dilute H_2SO_4.			
25 c. c.	0·2620 gram.	0·1357 gram.	-----	0·5428 gram.	2·71
		Total carbohydrates A.			
25 c. c.	0·1210 gram.	0·0616 gram.	-----	0·4928 gram.	2·46
		Total carbohydrates B.			
25 c. c.	0·1410 gram.	0·0718 gram.	-----	0·5744 gram.	2·87
		Total carbohydrates C.			
25 c. c.	0·1405 gram.	0·0715 gram.	-----	0·5720 gram.	2·86

	A. Without peptones, 1 hour 20 min. at 40° C.	*B.* Without peptones, 1¼ hours at 40° C. and 16 h. at 20° C.	*C.* With peptones, 1¼ hours at 40° C. and 16 h. at 20° C.
Glycogen	0·44 per cent.	0·22 per cent.	0
Sugar before boiling with H_2SO_4	1·94	2·52	2·39 per cent.
Sugar after boiling with H_2SO_4	2·15	2·62	2·71
Total carbohydrates	2·46	2·87	2·86

A comparison of these results shows no increase whatever in total carbohydrates in the presence of peptone (*B* and *C.*) Somewhat strange is the apparent increase of 0·41 per cent., in the absence of peptone after standing 24 hours. The percentage of glycogen is diminished one-half after standing 24 hours, and, as has been noticed several times before, it is still further diminished in the presence of peptone. Consistent with the theory of the formation of liver sugar from the hepatic glycogen, is the fact that in this experiment increase in sugar accompanies decrease in glycogen. This is shown best in the percentages of sugar, after boiling with dilute sulphuric acid. The relative reducing power of the sugar solutions, before and after boiling with dilute acid, is in *A* 90·1 : 100, in *B* 96·4 : 100, and in *C* 88·3 : 100. In other words the liver sugar in *B* after standing 24 hours is composed mainly of dextrose, the lower reducing body present in larger quantity in *A* having been gradually changed by longer contact with the liver tissue. In *C*, however, although the time was the same as in *B*, the presence of peptone, while it appears to increase the decomposition of glycogen and thus the actual amount of sugar formed, tends to prevent apparently the conversion of the lower reducing sugar into dextrose; hence in *C* the percentage of sugar before boiling with acid is less than in *B*, while after treatment with acid it is greater.

Following is a resumé of the various results obtained with peptones.

Experiment I.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood.	5·46 %	2·91 %	11·08 %
40	Without " "	6·21	2·74	10·75
	2 hours at 40° C.	−0·75	+0·17	+0·33

Experiment II.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood.	7·46 %	3·26 %	14·15 %
40	Without " "	8·09	2·75	13·55
	2 hours at 40° C.	−0·63	+0·51	+0·60

Experiment III.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
25 grams.	With peptones and blood.	1·65 %	---	6·42 %
25	Without " "	1·54	2·86 %	5·84
	2 hours at 40° C.	+0·11		+0·58

Experiment V.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones.	3·51 %	4·39 %	10·17 %
50	Without "	3·28	4·46	9·37
	3 hours at 40° C.	+0·23	−0·07	+0·80

After boiling the sugar with dilute H_2SO_4, 4·39=5·82 % Ratio 76·4:100.
" " " " 4·46=5·75 " 78·1:100.
+0·07

Experiment VI.—Rabbit's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones.	6·46 %	3·49 %	13·48 %
40	Without "	5·84	4·23	12·90
	24 hours at 18–20° C.	+0·62	−0·74	+0·58

Experiment IX.—Cat's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones and blood.	0	1·74 %	2·13 %
50	Without " "	0	1·67	1·89
	2½ hours at 40° C.		+0·07	+0·24

After boiling the sugar with dilute H_2SO_4, 1·74=1·77 % Ratio 98·8:100.
" " " " " 1·67=1·79 " 93·6:100.
−0·02

Experiment X.—Cat's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
40 grams.	With peptones and blood.	0	----	2·72 %
40	Without " "	0	----	2·20
	2 hours at 40° C.			+0·52

Ratio before and after boiling with dilute H_2SO_4, 70·6:100, with peptones.
" " " " 75·4:100, without peptones.

Experiment XI.—Lamb's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones.	0	2·52 %	2·41 %
50	Without "	0	2·51	2·46
	4 hours at 40° C.		+0·01	−0·05

After boiling the sugar with dilute H_2SO_4, 2·52=2·57%
" " " " 2·51=2·52

Experiment XII.—Sheep's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones.	0	1·10 %	1·53 %
50	Without "	0	0·84	0·96
			+0·26	+0·57
	1½ hours at 40° C.			

Experiment XIII.—Calf's liver.

Liver taken.	Treatment.	Glycogen.	Sugar.	Total carbohydrates.
50 grams.	With peptones.	0	2·39 %	2·86 %
50	Without "	0·22 %	2·52	2·87
	1½ hours at 40° C. and			
	16 hours at 20° C.	−0·22	−0·13	−0·01

After boiling the sugar with dilute H_2SO_4, 2·39=2·71 % Ratio 88·8:100.
" " " " 2·52=2·62 " 96·4:100.
+0·09

These results show throughout no indications whatever of a formation of sugar from peptones; on the contrary the results are wholly in accord with the formation of liver sugar from the hepatic glyco gen. At the same time it is apparent in several cases, that the decrease of glycogen is somewhat in excess of the increase in sugar, which fact would agree with the view of Seegen and Kratschmer that the destroyed glycogen is consumed in some other manner than by conversion into sugar. But this is in the liver after death, from which we cannot assume like action during life. There is further, in the results obtained, strong evidence that the liver sugar, as found after death, is a mixture of maltose and dextrose; the former presumably being converted into the latter by further ferment action. As to the total carbohydrates the results certainly accord in a general way with those obtained by Seegen. In nearly every case, the presence of peptone gives rise to an increase in total carbohydrates; the average increase in 8 experiments is, however, but 0·52 per cent. This increase, though small, is certainly too large to be explained by the assumption of analytical errors, and the increase, moreover, is too constant to admit of such an explanation. Futhermore, it is to be noticed in several instances, that in the presence of peptone the total carbohydrates found are greater than the sum of the boiled sugar and glycogen calculated as dextrose, while in the control they are practically equal; a fact which certainly lends favor to the view that the apparent increase of total carbohydrates is a real increase, due in some manner to the presence of peptone. Opposed to this view, however, or rather to the view that the liver possesses the power of

changing peptone into carbohydrates is the fact that in our experiments the same increase of carbohydrates is to be noticed after 24 hours' contact with peptone as after a shorter time; in fact in our experiments, time appears to have no noticeable influence on the total carbohydrates whatever, unless the like results in Experiment XIII are taken as confirmation of Seegen's statement that after a time the newly formed sugar is decomposed and thus the content of total carbohydrates falls back to its original amount; but in this experiment there is no evidence that the total carbohydrates ever were larger in amount. While our results therefore corroborate Seegen's and Kratschmer's statements regarding an increase of carbohydrates in the presence of peptone, we cannot consider that the slight increase in question, far less than that recorded in their experiments, is sufficiently pronounced to decide conclusively upon such an important theory. Furthermore, the noticeable lack of increase in sugar in the presence of peptone, excepting such increase as is attended with decrease in glycogen, is an additional reason for not attaching as much importance to the slight increase in total carbohydrates alone, as might otherwise be done. Consequently we must conclude that, in our opinion, the results obtained in these experiments do not warrant the adoption of this theory regarding the origin of the liver sugar, and that without further proof to the contrary we must still adhere to the formation of liver sugar from the hepatic glycogen.